Science Library

145.-

Vol. 67 NOVEMBER, 1940 No. 8

Bulletin

of the

Torrey Botanical Club

NOV 3 1940

Editor
Harold W. Rickett

Associate Editors
Edward W. Berry
M. A. Chrysler
Henry Allan Gleason
Tracy Elliot Hazen
Michael Levine
George M. Reed
William J. Robbins
John W. Shive
R. P. Wodehouse

CONTENTS

A Method for Describing and Comparing Blooming-Seasons. *Edgar Anderson and Leslie Hubricht* 639
Microsporogenesis in Triploid and Diploid Plants of Hemerocallis fulva ..... *Clyde Chandler* 648
Infection Studies on the Covered Smut of Oats ..... *Paul F. Brandwein* 675
Tar Spot of American Holly ..... *F. S. Luttrell* 692
Tegillum, a New Genus of the Uredinales ..... *E. B. Mains* 705
Characters for the Classification and Identification of Varieties of Capsicum ..... *H. L. Cochran* 710
Index to American Botanical Literature 718

Published for the Club monthly except
July, August and September
by
THE SCIENCE PRESS PRINTING COMPANY
LANCASTER, PENNSYLVANIA

Subscription price $5.00 per year

Entered as second-class matter October 8, 1940 at the post office at Lancaster, Pa., under the Act of March 3, 1879. Accepted for mailing at the special rate of postage provided for in the Act of February 28, 1925, embodied in paragraph 4, section 538, P. L. & R.

THE TORREY BOTANICAL CLUB

President
BERNARD O. DODGE, PH.D.
The New York Botanical Garden, Bronx Park, New York

Vice-Presidents
GEORGE T. HASTINGS, A.M.
E. B. MATZKE, PH.D.

Corresponding Secretary
JOHN S. KARLING, PH.D.
Columbia University, New York City

Recording Secretary
MISS CLYDE CHANDLER, PH.D.

Treasurer
HAROLD N. MOLDENKE, PH.D.
The New York Botanical Garden, Bronx Park, New York

Editor
HAROLD W. RICKETT, PH.D.

Bibliographer
MRS. LAZELLA SCHWARTEN

Business Manager
MICHAEL LEVINE, PH.D.

MEETINGS

Meetings twice each month from October to May, inclusive: the first Tuesday at 8:15 P.M., at the American Museum of Natural History or at Columbia University; the third Wednesday at 3:30 P.M., in the Museum Building of the New York Botanical Garden, Bronx Park, New York, N. Y.

PUBLICATIONS

All subscriptions and other business communications relating to the publications of the Club should be addressed to the Treasurer, Dr. H. N. Moldenke, The New York Botanical Garden, Bronx Park, New York, N. Y. Matters relating to advertisements and circulation should be referred to Dr. Michael Levine, Montefiore Hospital, New York, N. Y. Communications about exchanges should be sent to Dr. John S. Karling, Columbia University, New York, N. Y.

Bulletin. Monthly, except during July, August, and September; established 1870. Price, $6.00 a year; single numbers 75 cents. Manuscripts intended for publication in the Bulletin should be addressed to Dr. H. W. Rickett, The New York Botanical Garden, Bronx Park, New York, N. Y. The editors may accept papers up to 20 pages in length, with illustrations and tables generally not exceeding 20 per cent of the text. Longer and more copiously illustrated papers may be published if the excess is paid for by the contributors. Claims for missing numbers should be made to the Treasurer within 60 days of the date of mailing; they will be supplied free only if they have been lost in the mails. For back numbers write to the Treasurer.

Contributors may order reprints of their articles when they return galley proof to the editor. A schedule of charges is sent with the proof and will be supplied to prospective contributors on request.

Torreya. Bimonthly; established 1901. Price $1.00 a year. Manuscripts intended for publication in Torreya should be addressed to Dr. John M. Thomson, The American Museum of Natural History, New York, N. Y.

Memoirs. Occasional; established 1889.

Preliminary Catalogue of Anthophyta and Pteridophyta within 100 miles of New York City, 1888. Price, $1.00.

BULLETIN
OF THE
TORREY BOTANICAL CLUB

VOLUME 67 NOVEMBER · 1940 NUMBER 8

A METHOD FOR DESCRIBING AND COMPARING BLOOMING-SEASONS

EDGAR ANDERSON AND LESLIE HUBRICHT

(WITH SIX FIGURES)

For practical reasons connected with the management of the wild-flower reservation of the Missouri Botanical Garden's Arboretum at Gray Summit, Missouri, records of flowering-dates for about 100 species have been made there since September 1937. The species selected were those of particular interest to the public either because they made beautiful displays in the landscape (*Mertensia virginica, Coreopsis lanceolata,* etc.) or were particularly odd or interesting (*Spiranthes gracilis, Arisaema Dracontium,* etc.) (Anderson 1937). These 100 species were scored once a week throughout the blooming-season (so far as possible) and were recorded as: 'coming into flower,' 'in full bloom,' 'passing out of flower,' or 'out of bloom.' In those borderline cases when it was difficult to say whether a species should be recorded in one category or another, the decision was made with reference to its landscape effect. *Viola striata,* for instance, has the habit of producing a very few flowers long after the main blooming-period is over. It was scored as 'passing out of bloom' as long as it gave that general effect. When the flowers became so few that they could not be found without getting down and searching among the leaves, the species was scored as 'out of bloom.'

As the data began to accumulate it became apparent that they could be put to various uses in addition to the utilitarian ones for which they had been gathered. In particular it occurred to us that by assigning numerical values to the scores it would be possible to add up the totals for each week and in this way produce a picture of the rise and fall of bloom throughout the season.

Such a curve could be used to compare different blooming-seasons or to compare the season of bloom in one locality with that in another. Quantitative values were therefore assigned to the records: 'full bloom,' three; 'coming into bloom,' one; and 'going out of bloom,' two, these values being approximately proportional to the landscape effect of the three conditions.

[THE BULLETIN FOR OCTOBER (67: 565–638) WAS ISSUED OCTOBER 1, 1940]

The curve derived from these weekly totals is shown in figure 1, which gives a complete record for 1938 and such data as are available for 1937 and 1939.

As is shown in figure 1 the blooming-season at Gray Summit begins about the first of March and rises sharply for about eight weeks, then declines almost as rapidly for another eight weeks. After a two-week lull it increases slowly to another peak in midsummer. In favorable seasons the falling off from this peak is halted and the curve may even rise for a week or two when the autumnal Compositae come into bloom in September. There is in other words a sharp spring peak and a broader one in late summer separated by a distinct lull in late spring and early summer.

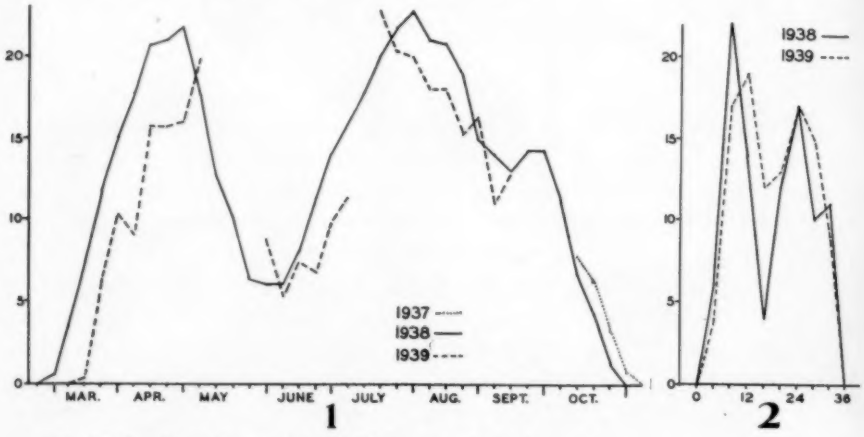


FIG. 1. Number of species in full bloom each week.
FIG. 2. Number of species at peak of bloom, by four week periods.

While the pronounced division into spring and summer bloom in Missouri has doubtless been noted by other observers, the only printed reference which has come to our notice is by Mrs. Rickett (1937, p. 69).

While the graph in figure 1 is based on roughly one-sixth of the species of native flowering plants, it is apparently a random selection, so far as blooming-dates are concerned. Certainly the large groups of plants that were not recorded present the same general features. The Amentiferae, for instance, would all coincide with the spring peak, the Cyperaceae and the Gramineae have high spring peaks and lower ones in late summer.

The curve shown in figure 1, while it presents an accurate picture of the wealth of bloom throughout the season, is undesirable for purposes of analysis. It is the resultant of two different effects; (1) the number of species coming into flower throughout the season and (2) the length of time these species remain in bloom. As can be seen from the original data in table 1, length of bloom not only varies from species to species but the average length of bloom in the summer is much longer than that in spring. By adapting a

suggestion of Prof. N. C. Fassett it was possible, however, to separate these two effects, and the curves shown in figures 2, 5, and 6 show the seasonal progression of number of species without reference to the length of time they remain in bloom. This was done by determining the middle of each species' blooming-period and graphing the number of these in every four-week period throughout the season.

The corrected curves for 1938 and 1939 are shown in figure 2 (the incomplete data for 1939 have been supplemented by estimates derived from the 1938 data and from observations made in previous years). It will be seen that the corrected curve presents the same general picture of spring and summer peaks of bloom separated by a less active period in early summer and that the curves for the two years are quite similar.

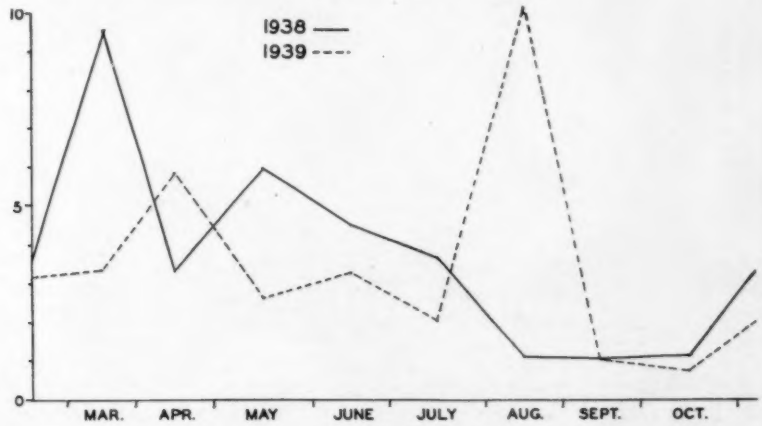


FIG. 3. Inches of rainfall at St. Louis, Mo., by months.

It is one thing to demonstrate such a curve but quite another to analyze the forces behind it. For the present we shall do no more than to outline some of the complexities involved in its correct interpretation. It is certainly affected by both environmental and inherent factors.

The effect of the environment is difficult to interpret because it is dual, the immediate effect, and its effect through natural selection. Both of these are particularly evident in the blooming-dates of those species which flower in the spring woodlands. The spring peak coincides with the time when the majority of trees are coming rapidly into full leaf and most of the bloom in the spring woods is over by the time the leaves are fully expanded. The spring flowers come on with a rush, as if in a hurry to blossom and go to seed while the woodlands are still sunny. However, they blossom in the same way and at the same time if transplanted to full sun. Evidently, therefore, these species have evolved in woodland habitats and have for generations

TABLE 1

Data on blooming-seasons of 96 species growing at Gray Summit, Mo., arranged according to the three major habitats; glades, woods, and meadows. Symbols under 'Origin' indicate probable center of distribution: C = Coastal Plain; I = Interior Plateaus; O = Ozark-Texas; and X = Uncertain. Each species was scored once each week: Blank = not in flower; 1 = coming into flower; 2 = passing out of flower; 3 = in full flower. Further explanation in the text.

	Origin	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
GLADES									
Allium stellatum	O						1 3 3	3 3 3 3	2
Nothoscordum bivalve	C	1 3	3 3 2						
Camassia scillioides	I		1 1 3 2						
Agave virginica	C				1	3 3 3 3 3	3		
Nemastylis acuta	O		1 3 3						
Spiranthes gracilis	I					1	3 3 3 2		
Spiranthes cernua	X							1 3 3 3	3 3 2 2
Arenaria patula	I		1 3	3 3 3					
Clematis Fremontii	O		1 3	3 2					
Delphinium carolinianum	X			1 3 3 3	3 2				
Leavenworthia uniflora	I		1 3 2						
Petalostemum purpureum	I				1 3	3 3 2			
Oenothera missourensis	O			1 3 3 3 2	2				
Asclepias tuberosa	C				1 3 3 2	2			
Asclepias verticillata	C				1 3	3 3			
Heliotropium tenellum	O					1 3 3 3 3	3 3 3		
Lithospermum canescens	O	1	3 3 3 3	3					
Scutellaria parvula	I			1 3 3 3 2	2				
Ruellia caroliniensis	C				1 3 3	3 3 3 3 3	3 3 3 2	2	
Houstonia angustifolia	C				1 3 3	3 3 3 3 3	3 3		
Vernonia crinita	O					1	3 3 3 2		
Liatris cylindracea	O					1	3 3 3 3	3 3 2	
Liatris pycnostachya	O					1 3 3			
Aster oblongifolius	I						1 1 1	1 1 1 1	3 3 3 3 2
Silphium laciniatum	O				1 3	3 3 3 3 3	3		
Rudbeckia missouriensis	O					1 1 3 3	3 3 2 2		
Echinacea pallida	O			1 3 3	3 3 2				
Coreopsis lanceolata	C			1 3 3 3 2	2				

TABLE 1.—(*Continued*)

TABLE 1.—(*Continued*)

	Origin	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
Woods									
Arisaema triphyllum	I	1	3 3 3 3	3 2					
Arisaema Dracontium	I		1 3 3 3	3 3					
Tradescantia virginiana	I		1	3 3					
Tradescantia canaliculata	C			1 3 3 3 2					
Uvularia grandiflora	I		1 1 3 2						
Erythronium albidum	I	1 3 2				1 3 3	3 2		
Hexalectris spicata	C				1	3 3 3			
Silene stellata	I								
Claytonia virginica	I	1 3 3 3	2 2 2						
Isopyrum biternatum	I	1 3	3 3 2						
Aquilegia canadensis	I		1 3 3 3	3 3					
Delphinium tricorne	I	1	1 1 3 3	3					
Sanguinaria canadensis	I	1 3 3 2							
Iodanthus pinnatifidus	I			1 3 3 3					
Dentaria laciniata	I	1 3 2	2						
Amelanchier canadensis	I	1 3							
Cercis canadensis	I	1 3	3 3						
Astragalus distortus	O		1 3 3	3 3 2 2					
Viola pedata	I	1	3 3 3 3	2					
Viola striata	I	1	3 3 3 3	3 2 2 2 2					
Viola papilionacea	I	1 3 3	3 3 2 2						
Cornus florida	I		1 1 3 2	2					
Dodecatheon Meadia	I		1 3 3	3 2					
Phlox divaricata	I	1	1 3 3 3	2					
Phlox paniculata	I					1 3 3 3	2		
Polemonium reptans	I	1	3 3 3 2						
Hydrophyllum appendiculatum	I		1 3	3 2 2					
Phacelia Purshii	I		1 3	3					
Mertensia virginica	I	1	3 3 2						
Monarda Bradburiana	O		1	3 3 3 2					
Veronicastrum virginicum	I				1	3 3 3 3 2			
Viburnum rufidulum	I		1 3	3					
Campanula americana	I					1 3 3 3	3 3 2		
Lobelia siphilitica	I					1	1 1 3 3	3 3 3 3	3 2
Eupatorium urticaefolium	I					1 3	3 3 3 3	3 3 3 3	2

TABLE 1.—(*Continued*)

	Origin	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
WOODS (*Continued*)									
Eupatorium coelestinum	C						1 1 3	3 3 3 2	2 2
Solidago ulmifolia	I					1 3	3 3 3 3		
Aster patens	I							1 3 3	3 2 2
Aster turbinellus	O						1	1 1 3 3	3 3 2
Aster anomalus	O							1 1 3 3	3 3 2
Aster Drummondii	O							1 3	3 3 2 2
Aster sagittifolius	I							1 3	3 3 3 2 2
Rudbeckia triloba	I					1 1 3	3 3 3 2	2	
Rudbeckia laciniata	I					1	3 3 3 3	2	
Ratibida pinnata	I				1 3	3 2 2 2			
MEADOWS									
Iris virginica	C			1 3 3 2					
Rosa setigera	I				1 3 3 3				
Cassia fasciculata	C					1 3 3 3	3 3 2		
Hibiscus militaris	C					1 3	3 3 3 3	2	
Sabatia angularis	C					1 3 3 3 2			
Amsonia illustris	O		1	3 3 2					
Physostegia virginiana	I						1 3 3 3	3 3	
Monarda fistulosa	I				1	1 3 3 3 2	2		
Penstemon Digitalis	I		1	3 3 3 3 3	3 2 2				
Gerardia tenuifolia	I						1 3 3	3 3 2 2	
Campsis radicans	C				1 3	3 3 3 3 3	3 3 3 3	2 2	
Cephalanthus occidentalis	C					1 1 3 3 2	2		
Houstonia minima	O	1 3 3	3 2						
Vernonia altissima	I				1	3 3			
Vernonia Baldwini	O					1 3 3	3 3 2		
Solidago altissima	I						1	1 3 3 3	2 2
Solidago serotina	I						1 1 3	3 3 3 3	3 2
Aster pilosus	X						1 1 1	1 1 3 3	3 3 2 2
Aster vimineus	I							1	3 3 2 2
Silphium perfoliatum	O					1 3	3 3 3 3		
Rudbeckia hirta	I				1 3 3 3	3 2 2 2			
Helianthus sp.	X							1 1 3 3	2
Bidens polylepis	O						1 3	3 3	

been selected towards a type which could finish its blooming season by this time.

This dual effect of the habitat on blooming season is shown in figures 5 and 6. In figure 5 the species used in drawing the curves of figure 2 are graphed according to the habitat in which they occur at Gray Summit and separate curves are drawn for each. For this purpose the habitats are grouped as woods, meadows, and glades. The last of these terms follows local usage in the Ozarks and indicates a peculiar and interesting habitat which has not yet received the scientific study it deserves. In Missouri a glade designates a rocky treeless area from a few square feet to several acres

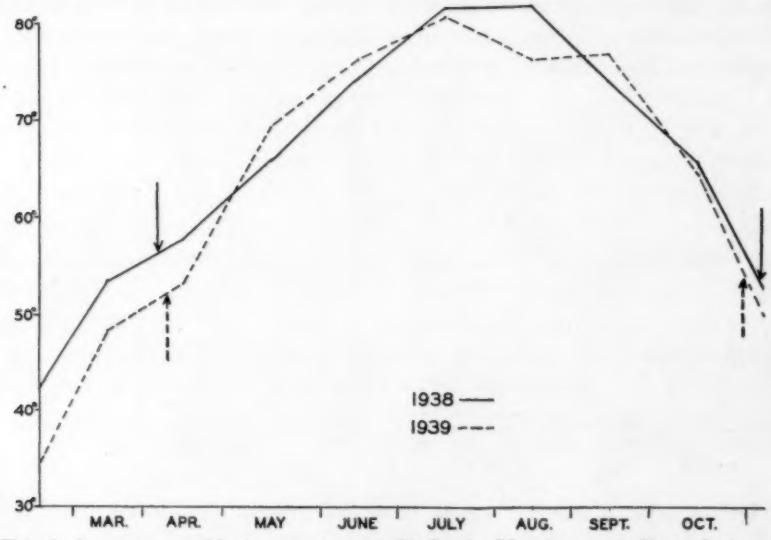


FIG. 4. Average monthly temperature at St. Louis, Mo. Arrows indicate last and first killing frosts.

in extent, somewhat analogous to the cedar barrens of Kentucky and Tennessee studied by Harper (1926) and Freeman (1933). The glades are even more closely allied to central parts of the Edwards Plateau and the Arbuckle Mountains. They are cold in winter, wet with seepage water in late winter and spring, and hot and dry throughout the summer.

It will be seen from figure 5 that the seasonal rhythm of bloom is radically different in the three habitats. The woodlands have the bulk of bloom in the spring, approach zero in early June and develop a lesser season of bloom in the late summer and fall. The treeless habitats are under no such pressure to blossom in early spring. Meadow-bloom rises rather slowly and more or less continuously to its peak in mid-summer. The glades are later to respond than either of these habitats and then maintain a fairly even amount of bloom throughout the year. Both the meadows and the glades.

however, show a drop in early June, though it is not so marked as that shown by the woodlands.

In figure 6 the species have been grouped according to the general character of their distributions. For this purpose distribution maps were prepared for each species. Inspection of these showed at least three main types of distribution (1) species centered upon the Gulf Coastal Plain (2) species centered on the Ozark-Texas axis and (3) species centered upon the interior plateaus of the Ohio-Mississippi River Basin (Anderson and Hubricht, unpublished). Most of the distributions could easily be placed in one of these three general categories, though a few had to be more or less arbitrarily assigned. The category to which each species was assigned is indicated in table 1. Some of the species are so poorly understood, taxonomically, that their distributions reflect their heterogeneity, and they have been omitted in

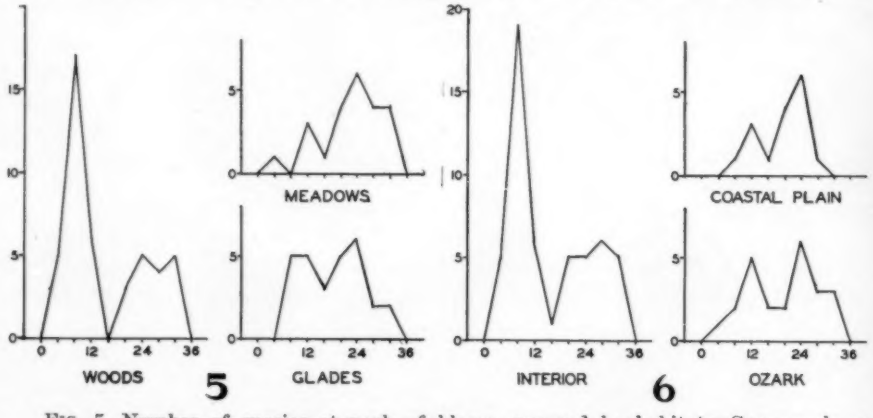


FIG. 5. Number of species at peak of bloom, grouped by habitats. Same scale as fig. 2.

FIG. 6. Number of species at peak of bloom, grouped according to probable center of distribution. Same scale as fig. 2.

preparing figure 6. It will be seen that the curves of figure 6 show the same general features as figure 5, though the two peaks of bloom are even more sharply revealed. A study of the data in table 1 will show that the groupings of figures 5 and 6 are very similar; most of the species of the central interior plateaus occur in the woodland, the glades are populated very largely by species from the Ozark-Texas axis, etc. Unfortunately the number of species for which we have information is too small to break up the curves still further in an attempt to distinguish between the direct and the selective effect of the habitats.

While the curves of figures 5 and 6 seem to give a rational though incomplete explanation of spring blooming-periods, they offer no clue as to why the summer peak should have the form that it does. The woods are

certainly as full of light in early June as in late July. Why then should we find *no* conspicuous woodland flowers in June but five or six species in July? Nor is there any evident correlation with either the temperature (fig. 4) or the rainfall (fig. 3). The most likely explanation would be that it is due to some kind of interaction with length of day. The last two decades have seen great progress in the interpretation of blooming-dates with regard to length of day (Garner 1937). It is now known that there are at least two fundamentally different kinds of plants: 'short day' and 'long day.' Since the spring peak occurs during the period when days are lengthening, and the summer peak during that when they are shortening, it would seem that length of day is one of the factors involved. However, the maxima on the curve do not bear any simple relation to day length. The spring peak, for instance, does not in any way coincide with the greatest length (June 21) or with the greatest increase in length (March 21). If anything it comes closer to the latter than to the former and it may be that in a longer growing season the maxima would fall at the periods of maximum increase and decrease (March 21 and September 21). Obviously this is not a question which can be settled by records of blooming-dates from a single latitude. When similar statistics have been presented for other species and for other localities it may then be possible to discuss the phenomena authoritatively. It is with the hope that comparable records may be collected elsewhere on some of these same species that the original data for 1938 have been presented in full in table 1. It would seem probable that if a group of these same species could be studied in the north they might show a single peak of bloom. Farther south one would expect an even wider division between peaks than is found in Missouri.

SUMMARY

1. A method is described for combining data on blooming-periods from various species to produce a generalized curve for an entire locality.

2. By the use of this method the blooming-season of 100 species native to Gray Summit, Missouri, was analyzed for 2 successive years. In their main features the 2 curves were similar. Each showed (a) a sharp peak of bloom in the spring (b) an almost equally abrupt cessation of bloom in early summer (c) a broad peak of bloom in late summer and autumn.

3. The interpretation of the factors responsible for these three features are discussed and the interplay of both environmental and inherent factors is suggested.

4. As a first step in analysis the above curve is broken down into separate curves for the three main types of habitat in the area and for the three chief distribution centers characterizing the species.

It is shown that the data from this one locality do not provide critical

evidence for evaluating the roles played by environment and germplasm in the determination of blooming-seasons.

5. The actual data are presented in tabulated form in the hope that comparable statistics may be collected for some of these same species at other localities.

THE MISSOURI BOTANICAL GARDEN
AND
WASHINGTON UNIVERSITY
ST. LOUIS, MISSOURI

Literature Cited

Anderson, Edgar. 1937. The development of the wild-flower gardens at the Arboretum. Bull. Mo. Bot. Gard. **25**: 129–139.

Freeman, Chester P. 1933. Ecology of the cedar glade vegetation near Nashville, Tennessee. Jour. Tenn. Acad. Sci. **8**: 143–225.

Garner, W. W. 1937. Recent work on photoperiodism. Bot. Rev. **3**: 259–275.

Harper, Roland M. 1926. The cedar glades of middle Tennessee. Ecology **7**: 48–54.

Rickett, Theresa C. 1937. Wild flowers of Missouri. Mo. Agr. Ext. Circ. 363: 1–144.

MICROSPOROGENESIS IN TRIPLOID AND DIPLOID PLANTS OF HEMEROCALLIS FULVA

CLYDE CHANDLER

(WITH SIXTY-ONE FIGURES)

INTRODUCTION

The object of this study is to compare the old and widely cultivated triploid clone, *Hemerocallis fulva* clone Europa, with wild diploid plants of this same species in respect to (1) the identity and structure of the chromosomes in each genom; (2) the behavior of these chromosomes in microsporogenesis; and (3) the extent and character of the various irregularities which result in the abortion of microspores.

Triploid clones in cultivation (Stout 1932) comprise: 1. the single-flowered (a) Europa Daylily and (b) the Maculata Daylily; 2. the double-flowered types known under the names of (a) Kwanso, (b) Flore Pleno, and (c) Variegated. All these plants are similar in habit of growth and color of flowers and all obviously belong to the somewhat variable wild species *H. fulva*.

The cytological studies already made of *H. fulva* may be grouped as follows:

1. Nearly all studies have been on *H. fulva* clone Europa. Many authors, e.g., Tangl (1882), Strasburger (1882), Biourge (1892), Juel (1897), Fullmer (1899), Schurhoff (1913), Tischler (1915), Belling (1925), Yamaha (1926), and Stout and Susa (1929), have studied plants of this clone and have been chiefly concerned with the numerous irregularities which occur during meiosis. Belling (1925) recognized that this clone is a triploid, and this condition affords a new interpretation of the earlier observations of much irregularity during meiosis.

Takenaka (1929) made studies of somatic cells in root tips of a plant which he called *H. fulva* and found the triploid number 33 chromosomes. His plant may or may not have been a member of the clone Europa.

2. A few studies have been made with other triploid clones of this species. Takenaka (1929) reported triploidy in a double-flowered clone which he called "*H. disticha* var. Kwanso." This is one of the old cultivated clones.

Another clone commonly designated as Flore Pleno was studied by Sienicka (1929). She reported irregular nuclear behavior during meiosis which results in polyspory. Unequal distribution of chromosomes at first division was recognized; however the chromosome number was not determined for this clone.

3. Some studies have been made of diploid plants. Takenaka (1929)

found the diploid number 22 in plants of "*H. disticha* Donn," and "*H. longituba,*" both of which evidently are to be included in the species *H. fulva* (Stout 1934).

Dark (1932) studied a diploid plant of *H. fulva* which he considered to be of the type from which the triploid *H. fulva* clone Kwanso probably arose. Of the eleven chromosomes in a single genom, Dark distinguished three pairs of long chromosomes with median or sub-median attachment constrictions and from two to four pairs with sub-terminal attachments. In no other paper has an attempt to identify the chromosomes in any plant of *H. fulva* been reported.

4. Stout (1932) reported the result of cytological studies made on seedlings obtained by crossing the triploid fulvous daylilies, clone Europa and clone Maculata, with certain diploids. The majority of these seedlings were diploid, a few were triploid, and several were aneuploid with somatic numbers which fluctuated between the diploid and the triploid.

It is thus well established that the species *Hemerocallis fulva* comprises plants with a diploid number of 22 chromosomes and plants with a triploid number of 33 chromosomes.

The diploid number (22) has been well established in all the other species of *Hemerocallis* which have been investigated (Stout 1932, Takenaka 1929, Dark 1932, and Matsuura and Sutô 1935).

MATERIAL

The studies of the triploid condition to be reported in this paper are of the widely cultivated clone of the single-flowered fulvous daylily which was in cultivation in Europe as early as 1576 (Lobel 1576) and which has been propagated by vegetative means only. These plants are referred to as "*H. fulva* clone Europa" (Stout 1932). This clone is so completely self-incompatible that its members produce no seeds to self, close, or intra-clonal pollinations. Only a small percentage of the pollen formed is viable. The results of numerous hybridizing pollinations indicate that very few of the ovules are able to form seed in seed production. More than 5 seeds have never been obtained in any capsule (Stout and Chandler 1933). There are, then, two distinct types of sterility in the members of the clone Europa: (1) physiological incompatibilities in fertilization and (2) abortion of microspores, which is to be reported in detail in this paper.

In 1933 seeds of wild plants of *Hemerocallis fulva* were received from Albert N. Steward, who collected them near Lu Shan, Kiangsi Province, China. Plants grown from these seeds are now well established at the New York Botanical Garden, and it is from these diploid plants that material has been collected and studied for comparison with the triploid clone Europa.

METHODS

Aceto-carmine smears of pollen mother-cells were made according to Belling's method (1921), and made permanent by the method described by McClintock (1929). Some of these preparations have been kept in good condition for eight months by sealing them with a mixture of paraffin and gum mastic. The staining of the chromatin material in the early prophases was improved by adding a trace of Ehrlich's haematoxylin to the aceto-carmine, care being taken to prevent the formation of a precipitate.

Anthers from the young flower buds were fixed with Flemming's "medium" fluid, with Carnoy's, Nawashin's, Allen's modification of Bouin's, and chromo-acetic fixatives. Microtome sections were made from 7 to 24 μ in thickness. Flemming's triple stain, Heidenhain's haematoxylin, and Newton's gentian-violet-iodine combination were used. Feulgen's reaction was especially helpful in locating chromatin material often found in the cytoplasm outside the organized nucleus.

Smears (Warmke 1935, Heitz 1926) as well as paraffin sections of root tips were made. The fixatives and stains used for anthers were employed also for making permanent slides. Both cross and longitudinal sections were studied.

Mature pollen grains were prepared for study by the methyl green glycerine jelly method described by Wodehouse (1935).

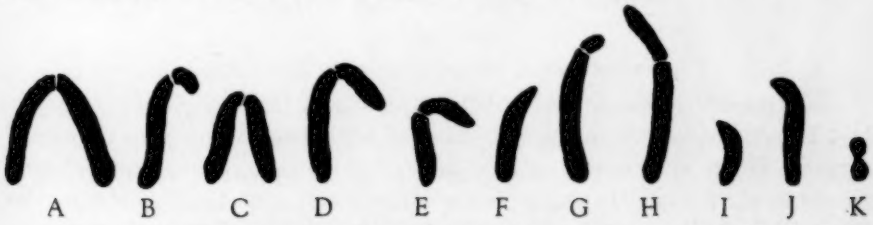


FIG. 1. Semi-diagrammatic drawings showing the relative sizes and shapes of the eleven chromosomes of a genom of *Hemerocallis fulva* clone Europa.

THE IDENTITY AND NUMBER OF THE CHROMOSOMES

The three sets of eleven chromosomes each in the triploid *Hemerocallis fulva* clone Europa may be identified in somatic mitosis (fig. 1). In all cells the three homologous chromosomes are so alike that I have not been able to distinguish between them. These studies were made from prepared slides of root tips fixed with Flemming's medium fluid and stained with Heidenhain's haematoxylin and Flemming's triple combination. Each of the eleven chromosomes of a set has been identified.

The data presented in table 1 may be conveniently summarized as follows:

Four of the chromosomes, A, C, E, and K, have median attachment

TABLE 1

The chromosomes of the type clone of Hemerocallis Europa

Chromosome	Length in μ	Insertion region
A	4.25–4.25	Median
B	5.1	Sub-terminal
C	3.4 –3.4	Median
D	4.25–2.5	Sub-median
E	2.5 –2.5	Median
F	3.7	Terminal
G	5.9	Sub-terminal
H	5.1 –2.5	Sub-terminal
I	2.5	Terminal
J	5.9	Terminal
K	2.2	Median

regions, A being the largest and K the smallest. C and E can be identified only by careful measurements. Both D and H have sub-median insertion regions. The body of H is longer than that of D. Chromosomes B and G have sub-terminal attachment regions, G being the larger. F, I, and J appear to have terminal fiber attachment regions and can be distinguished by measurements.

In the diploid, the two sets of eleven chromosomes were identified; each chromosome is identical in appearance with a chromosome of the triploid.

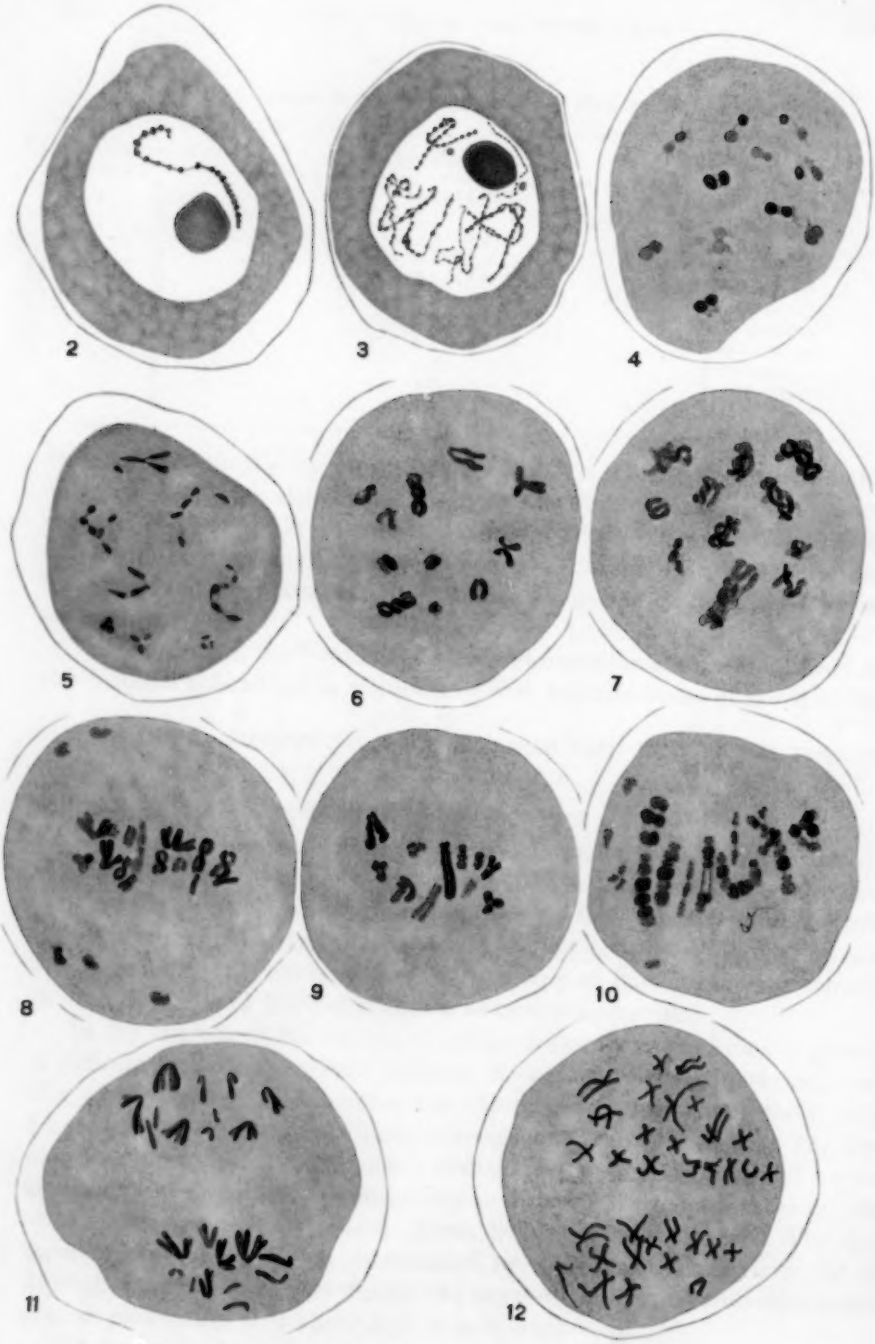
MEIOSIS IN THE DIPLOID ($2n = 22$) COMPARED WITH THAT OF THE TRIPLOID ($3n = 33$)

Prophases of the Heterotypic Division

In the early prophase stage in the diploid and triploid plants studied in this investigation, the nuclei are crowded with long fine zigzag chromatic threads which at a casual glance appear to form an irregular network. Closer study reveals that these threads are not continuous, but are a mass of single individual chromosomes. The pairing of homologous chromosomes in both diploid and triploid plants is always parasynaptic.

In the nucleus of the diploid at synapsis one large nucleolus is present. A definite nuclear membrane persists. Elongated chromatin threads, usually closely paired, chromomere for chromomere, throughout their entire length (fig. 2) are well distributed throughout the nuclear cavity. The chromomeres, first known as Pfitzner's granules (Pfitzner 1881) are of various sizes and appear spheroidal, but owing to their small size no further division of chromomeres into chromioles (Eisen 1899) has been observed. The chromomeres appear in a line surrounded by an achromatic matrix. Of the diploid it may be said that during the zygotene and pachytene stages there is close association of the homologs in each complement.

In the triploid the three homologs of the eleven chromosomal types have various degrees of association: (1) All three homologs may be rather closely



CHANDLER: TRIPLOID AND DIPLOID HEMEROCALLIS

associated and very few of the chromomeres fail to become associated with other chromomeres. Though few workers have reported this type of association, it has been observed by Belling (1929) who was of the opinion that all three homologous chromosomes of a triploid hyacinth become completely synapsed, the threads forming a trihedral filament with flattened angles. Similar associations have been found in a few other species. Skovsted (1933) found in triploid Asiatic cotton some conjunct triple threads. Olmo (1934) did not observe triple synapsis throughout the length of the chromosome in the triploid *Nicotiana tabacum* but reports triple synapsis for a "considerable" length of the chromosome. He found that three general regions of the chromosome seem to favor triple synapsis: the large terminal chromomeres, the spindle fiber attachment regions, and lengths of the chromonemata in which prominent chromomeres are closely adjacent. It becomes evident that the degree of association of homologous chromosomes during early prophase depends upon the type of triploid studied. It is to be expected that the associations of homologous chromosomes of an autotriploid may be more complete than those of allotriploids or triploids having less homologous chromosomes.

(2) Close pairing of two of the homologs with very loose pairing of the third was seen. This type of association has been described for the majority of the triploids studied by previous investigators.

(3) A third chromosome may remain completely unassociated with the other two of its group. This behavior has been observed also in triploid

Explanation of Figures 2–12

FIG. 2. Prophase of a pollen mother-cell of the diploid *H. fulva*, showing two homologous chromosomes synapsed at pachytene. Iron-haematoxylin. × 750.

FIG. 3. Prophase of a pollen mother-cell of the triploid *H. fulva*, showing the associations of three homologous chromosomes at pachytene. Iron-haematoxylin. × 750.

FIG. 4. Diakinesis in the diploid, showing eleven chromatin groups or eleven bivalents. Aceto-carmine. × 750.

FIG. 5. Diakinesis in the triploid, showing thirteen chromatin groups which include 10 trivalents and three univalents. Aceto-carmine. × 750.

FIG. 6. Late diakinesis in the diploid, showing eleven bivalents which are about to enter first metaphase. Aceto-carmine. × 750.

FIG. 7. Late diakinesis in the triploid, showing eleven trivalents just before the first metaphase. Aceto-carmine. × 750.

FIG. 8. Equatorial plate in the triploid, showing 5 univalents lagging at the periphery of the cell. Aceto-carmine. × 750.

FIG. 9. Equatorial plate in the diploid. Homologs separating previous to first anaphase. Aceto-carmine. × 750.

FIG. 10. Equatorial plate in the triploid; the chromatin material is organized in irregular masses which may later form a pycnotic nucleus. Aceto-carmine. × 750.

FIG. 11. First anaphase in the diploid; the chromatids of the eleven chromosomes at each pole separating for the second division. Aceto-carmine. × 750.

FIG. 12. First anaphase in the triploid with 19 chromosomes at one pole and 14 at the the other. The distribution of chromosomes is as follows: A, A, B, B, C, C, D, D, E, E, F, G, G, H, I, I, J, J, and K. Total 19. A, B, C, D, E, F, F, G, H, H, I, J, K, and K. Total 14. Aceto-carmine. × 750.

Nicotiana tabacum by Olmo (1934) and in triploid wheat by Horton (1936). In prophase at zygotene and pachytene univalent, bivalent, and trivalent association of the three homologs of the eleven chromosome types can be seen (fig. 3). Horton has proposed the term "amphispireme" for this stage of meiosis in which paired and unpaired chromatin threads exist. Multivalents of more than three chromosomes which might be the result of structural hybridity, translocations, or inversions were not seen. At diakinesis two cells were seen in each of which were ten trivalents and three univalents. Further observations would possibly reveal this type of association in the earlier prophases.

Diakinesis

In the diploid there are eleven pairs of chromosomes. No unpaired chromatic elements were observed. The homologous chromosomes, each composed of two tightly coiled chromonemata, are much shortened at this time. The association is, however, rather loose. They are not, as in some plants, arranged peripherally within the nuclear membrane, but are scattered throughout the nuclear cavity (fig. 4). One spherical nucleolus is generally present in each nucleus. One and sometimes two chromosomes are often closely appressed against the surface of the nucleolus; but many nucleoli show no association with any chromosome. Two nucleoli are sometimes present in a single nucleus. During the later stages of diakinesis chiasmata were observed for the longer chromosomes (fig. 6).

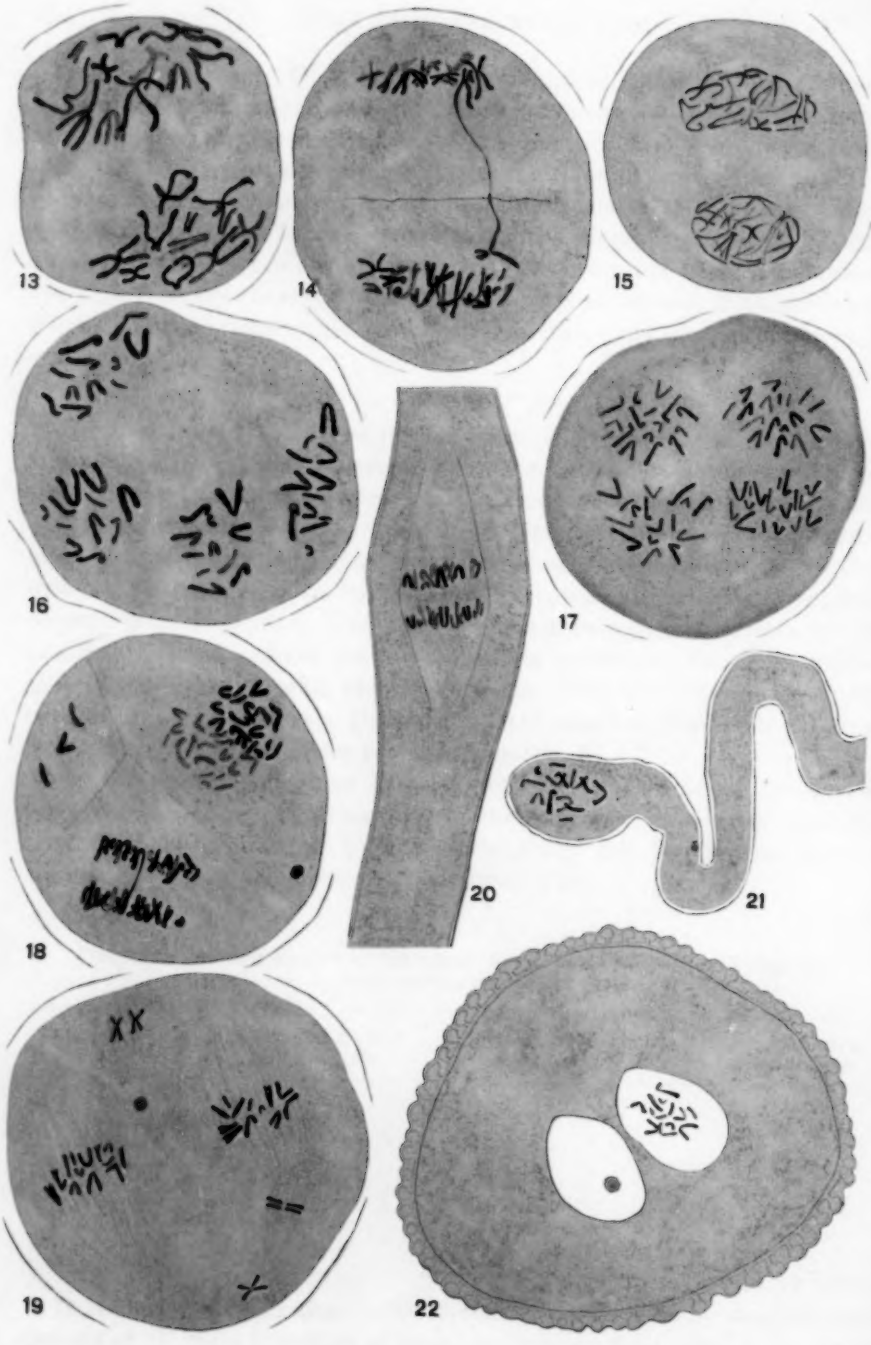
In the triploid the three homologs of each of the eleven chromosome types may be associated in any one of the four ways previously described. A study was made (table 2) of the frequency and distribution of these associations among the eleven chromosomal types.

TABLE 2

Frequency and distribution of the associations among the eleven chromosome types during diakinesis

Number of cells	Number and type of configurations	Total number of chromosomes	Number of chromatin masses
18	11‴	33	11
41	10‴–1″–1′	33	12
2	10‴– 3′	33	13
35	9‴–2″–2′	33	13
1	9‴–1″–4′	33	14
22	8‴–3″–3′	33	14
5	7‴–4″–4′	33	15
2	6‴–5″–5′	33	16

It is clearly evident that in 18 of the nuclei there were complete associations of all three homologs of each chromosome type (figs. 5, 7). In the other 118 cells the associations were less complete and resulted in the forma-



CHANDLER: TRIPLOID AND DIPLOID HEMEROCALLIS

UNIV. OF MICH.

tion of univalents, bivalents, and trivalents. In three cells in which all chromosomes were identified all three of the homologs of one of the chromosomal types remained completely unassociated.

In each cell at diakinesis 33 chromosomes were present; none had disintegrated into the cytoplasm. Occasionally a pycnotic nucleus was seen in which the chromosomes had lost all identity and the chromatin material appeared in a knotted mass. This is evidently the beginning of disintegration of the chromatin material in the earlier stages of meiosis. In no pollen mother-cell was chromatin observed which had been discharged into the cytoplasm before first division in the manner reported by Timm (1928).

Metaphase of the First Division

In the diploid eleven bivalents are arranged in the equatorial region (fig. 9). At this time the homologs are closely associated and no unpaired chromatin elements are to be found. The chromosomes are short and thick. Chiasmata are in evidence. The chromatids are so tightly coiled that no further division or duplication is here visible, although such division or duplication probably occurs.

In the triploid eleven trivalents have occasionally been seen in the equatorial region. Varying numbers of trivalents, bivalents, and univalents are more frequent. This agrees with the reports of Belling (1925) and Takenaka (1929). It is often difficult to interpret the associations of chromosomes at metaphase, as may be seen in figure 10.

Explanation of Figures 13–22

FIG. 13. First anaphase in the diploid, showing elongated chromatids completely separated except at the insertion region. Aceto-carmine. × 750.

FIG. 14. First anaphase in the triploid, showing chromatids stretched across the equatorial region between the two poles. Aceto-carmine. × 750.

FIG. 15. Early telophase in the triploid. Aceto-carmine. × 750.

FIG. 16. Second anaphase in the diploid with eleven chromosomes at each pole.

FIG. 17. Second anaphase in the triploid with 16 chromosomes at two poles and 17 at the the other two poles.

FIG. 18. Second anaphase in the triploid, showing three lagging chromosomes and a microcyst formed at first division. Fourteen chromosomes in one cell and 17 in the other have duplicated and separated.

FIG. 19. In the triploid at the end of second anaphase cells were seen in which the chromatids have not undergone second division but remained associated at their insertion regions giving the characteristic X-shaped structures. Lagging chromosomes may under division and often have accessory spindles of their own.

FIG. 20. The generative nucleus in the diploid divides after it passes into the pollen tube. Eleven chromosomes passing to each pole. Carnoy's, gentian violet. × 750.

FIG. 21. The generative nucleus in the triploid may divide in the tube. Thirteen chromosomes may be identified. Three A chromosomes and one each of the other types are present here. Carnoy's, gentian violet. × 750.

FIG. 22. The generative nucleus in the triploid may divide within the microspore. Twelve chromosomes may be identified. Two K chromosomes and one each of the other types are present here. Carnoy's, gentian violet. × 750.

As many as five univalents have been seen lagging in the cytoplasm (fig. 8). These probably never reach the equatorial plate. Irregular distribution of chromosomes to the poles may be due in part to this failure of univalents to become oriented on the spindle. This will be more fully discussed later.

Anaphase of the First Division

In the diploid the homologous chromosomes associated at metaphase separate and move toward the respective poles. At this time the chromatids of each chromosome begin to separate, except at the insertion region, for the second meiotic division. In figure 11 it may be clearly seen that one homolog of each pair of chromosomes passes to each pole, and when they are somewhat scattered all may be identified at each pole. No unequal distribution or lagging of chromosomes has been observed at the first division in the diploid. The chromatids appear elongated and the sister chromatids are widely separated except at the insertion regions (fig. 13).

In the triploid clone both unequal distribution and lagging of chromosomes are prevalent (fig. 12). All chromosomes were identified in ten cells at first anaphase (table 3). For these observations cells showing the least irregularity in chromosomal distributions were selected. As in the earlier

TABLE 3

Distribution of identified chromosomes at first division

Cells	Pole I												Pole II											
	A	B	C	D	E	F	G	H	I	J	K	Total	A	B	C	D	E	F	G	H	I	J	K	Total
1	2	1	2	2	2	2	2	1	2	1	1	18	1	2	1	1	1	1	1	2	1	2	2	15
2	2	1	2	2	1	1	2	1	1	2	1	16	1	2	1	1	1	2	1	2	1	1	1	14
3	3	2	1	1	2			2	2	1	3	17*		1	2	2	1	3	3	1	1	2		16
4	1	2	2	2	2	2	2	1	1	1	1	17	2	1	1	1	1	1	1	2	2	2	2	16
5	3	2	1	2	2	2	2	1		2	2	19		1	2	1	1	1	1	2	1	1	1	12
6	2		2	2	3	2	1		1	3		16	1	3	1	1		1	2	3	2		3	17
7	1	2	1	2	3		2	1	1		1	14	2	1	2	1		3	1	2	2	3	2	19
8	2	1	3	3		1	2	1				13	1	2			3	2	1	2	3	3	3	20
9	1	2	1	1	2	1	1	2	1	1	1	14	2	1	2	2	1	2	2	1	2	2	2	19
10	2	2	2	2	2	1	2	1	2	2	1	19	1	1	1	1	1	2	1	2	1	1	2	14

	Lagging Chromosomes												Grand Total
	A	B	C	D	E	F	G	H	I	J	K	Total	
1													33
2					1				1		1	3	33
3													33
4													33
5									2			2	33
6													33
7													33
8													33
9													33
10													33

* Chromatid stretched across equatorial plate from one pole to the other pole.

stages the three types of association between homologous chromosomes may be seen at this time. In nineteen cells the three homologs passed to the same pole. In two of these chromosome A behaved in this way; in one, chromosome B; in one, C; in one, D; in three, E; in two, F; in one, G; in one, H; in one, I; in three, J; and in three, K.

The distribution of chromosomes in other cells in which the chromosomes could be counted but not identified is shown in table 4.

TABLE 4

Distribution of unidentified chromosomes at first division

Pole I	Lags	Pole II	Total number of chromosomes	Number of cells
20	...	13	33	2
20	1	12	33	1
19	...	14	33	8
19	1	13	33	2
18	...	15	33	9
17	...	16	33	6
17	1	15	33	2
17	3	13	33	1
17	9	7	33	1
16	1	16	33	3

The study of 35 cells at the time when the chromosomes are passing to their respective poles during first anaphase shows that in most cells the chromosomes are rather evenly distributed to the two poles, the most frequent distributions being 18—15, 17—16, and 19—14.

Much irregularity in the distribution of chromosomes to the poles results from lagging chromosomes in the equatorial region. As many as nine have been observed about the equatorial plate after the other 24 chromosomes have moved to the poles. It is clearly evident that these different irregularities which end in abortion of microspores are not to be attributed to the aberrant behavior of any one chromosome. Evidently a mechanism which ordinarily functions effectively for the distribution of chromosomes into two equal groups when only two genoms are involved does not operate to distribute the chromosomes into equal groups when three genoms are present. As few as seven chromosomes may be seen at one pole. Further irregularities, such as the formation of the chromatin bridges previously noted in *H. fulva* by Juel (1897), Sienicka (1929), and Stout and Susa (1929), are not infrequent. Chromatin material may be stretched between the two poles, as seen in figure 14. It may be concluded that the chromatin bridges at the first anaphase result from the incomplete separation of chromatids due to the failure of terminalization of chiasmata.

A further study of 47 cells of the triploid at first anaphase and telophase

was made to determine the number of lagging chromosomes at first division. The numbers were as follows:

Number of Lags:	0	1	2	3	4	5	6	7	8
Number of Cells:	18	13	8	2	3	1	1	0	1

Twenty-nine cells (61.7 per cent) had lagging chromosomes. Eighteen cells (38.2 per cent) seem to have passed through first division without any loss of chromosomes or fragments of chromosomes. In the 13 cells in which a single chromosome was found lagging, various chromosomes have been identified. It is clearly evident that lagging is not a function of a particular chromosome.

The many irregularities in this triploid clone (*H. fulva* clone Europa) attracted the attention of earlier workers, such as Strasburger (1882). Takenaka (1929) reported unequal distribution of chromosomes at first anaphase, which gave "various combinations, e.g., 15, 18; 14, 18, 1; 14, 17, 1, 1; 16, 16, 1; etc." for the clone Kwanso. No mention of the frequency of the appearance of these various combinations was made.

During first anaphase it may be said that in the diploid there is an equal distribution of the chromosomes to their respective poles. No lagging was observed.

In the triploid unequal distribution of chromosomes to the poles, lagging chromosomes in the equatorial region, and chromatin bridges are prevalent.

Telophase of the First Division

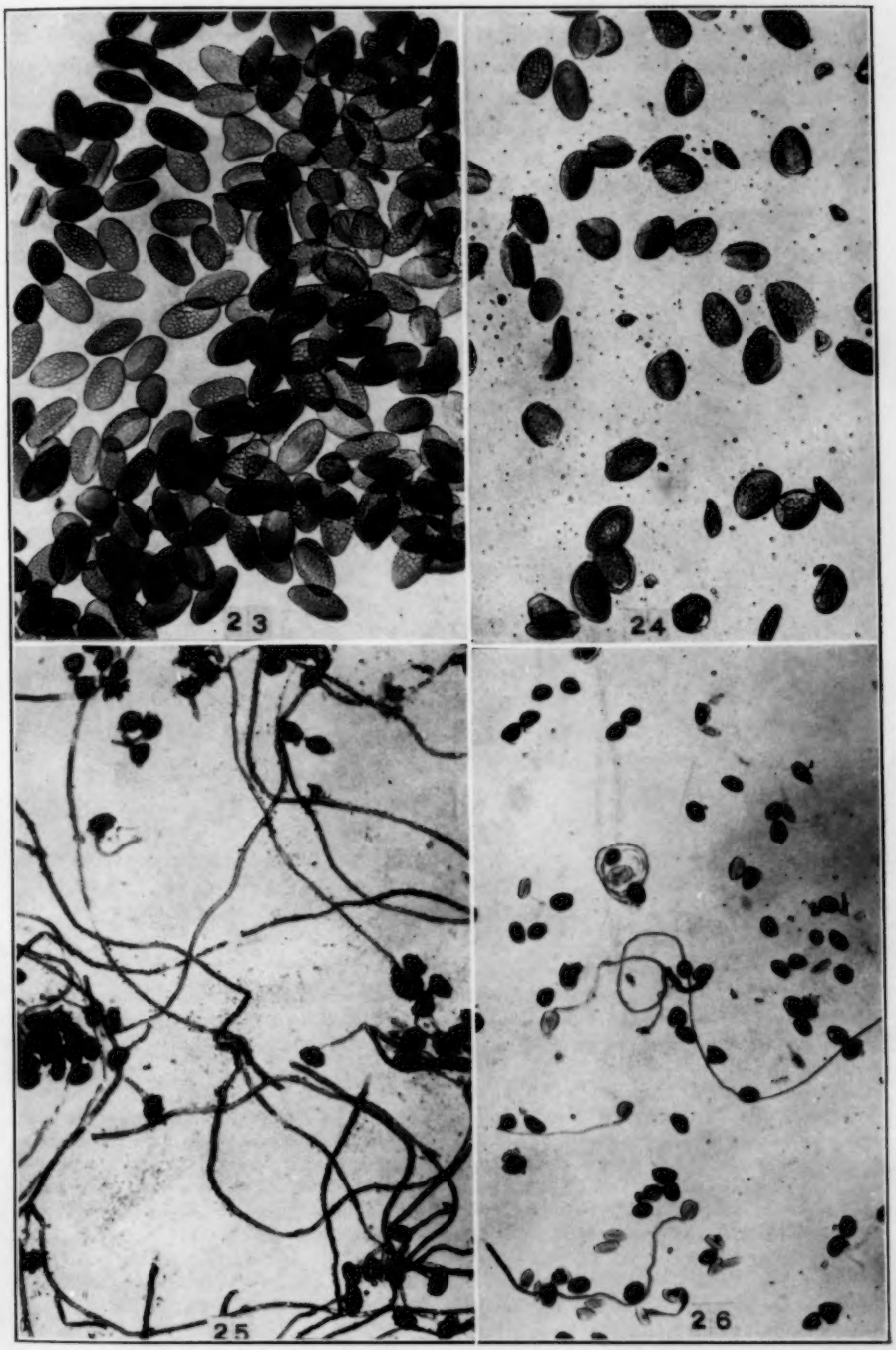
In the diploid shortly after the eleven pairs of homologous chromosomes reach their respective poles the coils of the chromonemata loosen and a nuclear membrane is formed around the chromosomes. A deeply staining partition is formed in the equatorial region of the spindle, but cytokinesis is not completed at this time (fig. 37).

In the triploid as many as seven daughter nuclei were observed, each connected with two others by spindle fibers as shown in figure 29. It is evident from studies made at second telophase that such a pollen mother-cell may not undergo further division. Similar figures have been seen in anthers in which most of the pollen mother-cells were in second telophase.

The chromosomes which lag may completely lose their characteristic form and appear as homogeneous material (microcysts) held in small vacuoles of the cytoplasm (figs. 33–37; compare *Lilium tigrinum* as reported by Chandler, Porterfield, and Stout 1937); or they may round up into small

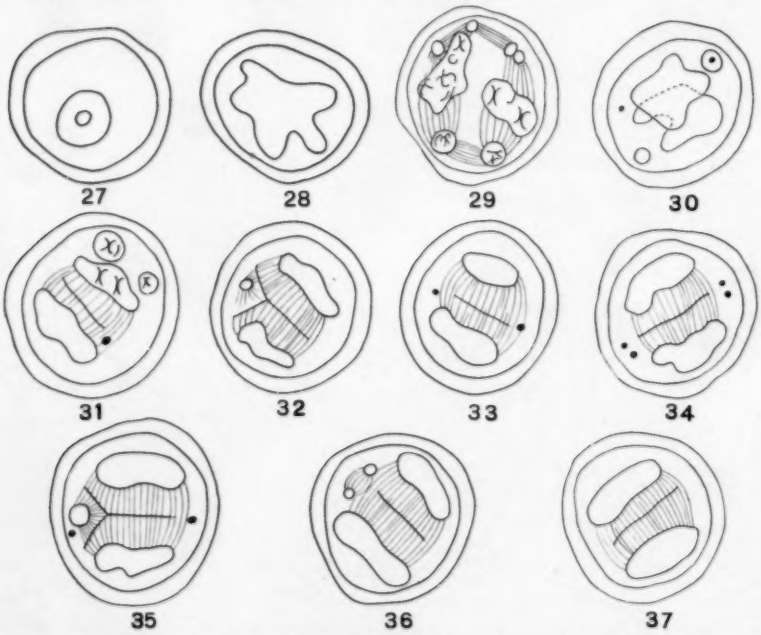
Explanation of Figures 23–26

FIG. 23. Mature pollen grains of the diploid. Methyl green glycerine jelly. × 85.
FIG. 24. Mature pollen grains of the triploid. Methyl green glycerine jelly. × 85.
FIG. 25. Germinating pollen of the diploid. Aceto-carmine. × 35.
FIG. 26. Germinating pollen of the triploid. Aceto-carmine. × 35.


23
24
25
26

nuclei and be included in the cytoplasm as microcytes (fig. 32), in which case they may retain recognizable form (fig. 31). As many as four microcysts have been observed in the cytoplasm of a pollen mother-cell at the end of the first division (fig. 34). Lagging chromosomes may also be arranged on a spindle of their own and divide simultaneously with the other chromosomes of the cell (fig. 36).

Pollen mother-cells with pycnotic nuclei have been occasionally observed (fig. 27). The nucleus may not divide, in which case it usually becomes irregular in shape (fig. 28), or at the end of the first telophase the two nuclei may be undulated (fig. 30). The same figure shows that both microcysts and microcytes may be found in the same pollen mother-cell.



FIGS. 27–37. Camera lucida drawings of pollen mother-cells at the telophase of the first division in the triploid. FIG. 27. Pycnotic nucleus in pollen mother-cells in which there was no division of chromatin. FIG. 28. Undulated nucleus which has undergone no division. FIG. 29. Pollen mother-cell in which more than two nuclei were formed as a result of lagging chromosomes. These nuclei may or may not undergo further division. FIG. 30. Pollen mother-cell with two irregular nuclei, one microcyst, and two microcytes, one of which contains chromatin. FIG. 31. Nuclei and micronuclei in a pollen mother-cell in which the chromosomes are X-shaped owing to the separation of the chromatids for the second division. FIG. 32. Two nuclei and one micronucleus separated by a deeply staining partition. FIG. 33. Two microcysts formed from lagging chromosomes. FIG. 34. Two large nuclei and four microcysts within one pollen mother-cell wall. FIG. 35. Two microcysts and a microcyte formed during first telophase in addition to the two main nuclei. FIG. 36. Pollen mother-cell showing a small spindle formed in the division of lagging chromosomes. FIG. 37. Two nuclei formed by equal distribution of chromosomes, with no lagging chromosomes.

Fullmer (1899) states that bodies with the appearance of centrosomes are frequently seen at the poles. It seems quite possible that these microcytes and microcysts are the bodies to which Fullmer referred.

Interphase

During interphase in both diploid and triploid plants the two nuclei are fairly large and the two chromatids of each chromosome appear as somewhat slender, crooked, and apparently single threads joined or held together at the spindle attachment region only. The chromosomes are peripherally arranged (fig. 15) and are parallel and somewhat appressed to the newly formed nuclear membrane. They become much dispersed before the second division ensues. The chromatin material now stains more faintly than at any other stage during meiosis.

The Homoeotypic Division

In the prophase of the second division the condensing chromosomes, in both the diploid and the triploid, appear characteristically in the form of threads or rods still associated in dyads at their attachment regions but diverging elsewhere, somewhat less widely than during the previous anaphase. By the end of the second prophase matrices have became conspicuous about the chromonemata, and as the spindle develops the chromosomes become oriented in the equatorial region. At this time the chromosomes are considerably shorter and thicker than at the end of the first anaphase.

In both the diploid and the triploid it may be noted that during metaphase the chromosomes are now much shortened and thickened. The two sister chromatids assume a more parallel position and soon separate completely.

In the triploid microcysts and microcytes formed from first division lags as described by Chandler, Porterfield, and Stout (1937) are plainly visible at the periphery of the equatorial plate region.

In the diploid the second anaphase is regular and eleven chromosomes pass to each pole (fig. 16).

In the triploid the two sister nuclei often have the same number of chromosomes in the second division, but owing to lagging chromosomes at second anaphase this number may be unequal. Chromosomes which lag at this time may become vacuolated and dispersed in vacuoles of the cytoplasm and form microcysts, or they may form microcytes like those formed from lags at the first division. It has also been observed that the difference in the number of chromosomes in sister nuclei cannot be interpreted on the basis of lagging chromosomes alone. It is evident that sister chromosomes may be distributed to the same pole.

Chromatin bridges were also seen in cells at the second anaphase, though less frequently than during first anaphase. These are for the most part

formed between two chromatids of two homologs during the anaphase of the first division, which have persisted through the second anaphase. They may be recognized by their orientation toward the partitions formed at first and second divisions as well as toward the four chromosomal groups. Chromosomes which lag for a time in the equatorial region may later move toward the pole. One end of the chromosome may be held in the equatorial region, thus stretching chromatin material from the equator to one pole. This is a purely mechanical process which does not correspond in any way to the processes ordinarily involved in the formation of generally recognized chromatin bridges. The separation of sister chromosomes at the second anaphase may not proceed with exact precision, in which case the complete separation of two of the sister chromosomes may be delayed. In such cells during early anaphase chromatin material is stretched across the equatorial region. These may or may not persist (fig. 18).

It is difficult to obtain satisfactory preparations for the study of the distribution of chromosomes at the second division, since slight pressure on the cover glass does not scatter the chromosomes as it does in the anaphase of first division. The chromosomes in one figure will often spread so that identification of individual chromosomes is possible, but seldom can one identify all the chromosomes of all four groups. However, all 66 chromosomes were identified in five cells. In ten other cells it was possible to count and study the distribution of the chromosomes to their respective poles. As many as eight lags have been found in the spindle region at the homoeotypic division. In some cells no chromosomes were found lagging at second anaphase (fig. 17).

The studies of the distribution of chromosomes at the homoeotypic division in the triploid are summarized in table 5.

Thirty-one other cells at second anaphase and early telophase were studied with respect to the number of lagging chromosomes, with data as follows:

Number of lags:	0	1	2	3	4	5	6	7	8
Number of cells:	5	3	7	3	9	2	0	0	2

Lagging chromosomes as shown in figures 18 and 19 were observed in at least 80 per cent of the pollen mother-cells studied. In no cell was an entire set of eleven chromosomes observed among the laggards.

In the diploid during telophase of the homoeotypic division a partition is formed in the region of the equatorial plate perpendicular to the long axis of the spindle, which may be parallel or perpendicular to the partition formed at the end of the heterotypic division (fig. 38). The nucleus assumes the resting condition, which is typical for the nucleus of the pollen grain.

In the triploid the telophase proceeds in the same way when only four nuclei are formed. When lagging chromosomes are present, a small nucleus

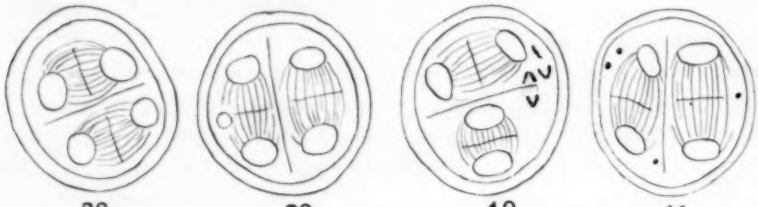
TABLE 5

Distribution of chromosomes at second division

Cells	Pole I												Pole II											
	A	B	C	D	E	F	G	H	I	J	K	Total	A	B	C	D	E	F	G	H	I	J	K	Total
1	1	2	2	2	2	1	1	1	2	2	2	18	1	2	2	2	2	1	1	1	2	2	2	18
2	1	1	2	1	2	2	1	1	3	1	2	17	1	1	2	1	2	2	1	1	3	1	2	17
3	1	2	1	1	1	2	1	1	2	2	2	16	1	2	1	1	1	2	1	1	2	2	2	16
4	2	1	2	2	1	2	1	2	2	1	1	17	2	1	2	2	1	2	1	2	2	1	1	17
5	2	1	2	2		1	2	1	2		1	14	2	1	2	2	2		2	1	2	1	1	16
6												15												18
7												17												17
8												17												17
9												17												17
10												16												16
11												14												16
12												18												18
13												16												16
14												21												21
15												15												14
	Pole III												Pole IV											
1	2	1	1	1	1	2	2	2	1	1	1	15	2	1	1	1	1	2	2	2	1	1	1	15
2	2	2	1	2	1	1	1	1		1	1	13	2	2	1	2	1	1	3	3		1	1	17
3	2	1	2	2	2	1	2	2	1	1	1	17	2	1	2	2	2	1	2	2	1	1	1	17
4	1	2	1	1	2	1	2	1	1	2	2	16	1	2	1	1	2	1	2	1	1	2	2	16
5	1	1		1	2	2	1		1	1		10	1	3	2	1	2	2	1	1	1	2	2	17
6												16												17
7												16												16
8												15												16
9												15												16
10												17												17
11												17												15
12												15												15
13												17												17
14												12												12
15												15												16
	Lagging Chromosomes												Grand Total											
1																								66
2										2		2												66
3																								66
4																								66
5							1		3		2	2	8											66
6																								66
7																								66
8																								66
9												1												66
10																								66
11												4												66
12																								66
13																								66
14																								66
15						1					1	2												66

may be organized about one or more of them and each lagging unit may retain its identity and become oriented in the cytoplasm, or it may lose its

identity, round up, and appear as a microcyst in the cytoplasm (fig. 30). By considering the position of the lagging units in the cytoplasm one can distinguish fairly accurately the laggards at first division from the laggards of second division (fig. 41). Chromosomes may remain in the cytoplasm without forming microcysts or microcytes and divide on small individual spindles at the end of second telophase (fig. 40). Microcytes may also be formed from chromosomes lagging at second division (fig. 39).



FIGS. 38–41. Pollen mother-cells at the telophase of the second division. FIG. 38. Four nuclei in a single pollen mother-cell. FIG. 39. Small nucleus formed from a second division lag, with the four nuclei usually formed. FIG. 40. A chromosome which lagged during first division and retained its individual form, dividing simultaneously with the other chromosomes in the cell. FIG. 41. Pollen mother-cell containing microcysts in the cytoplasm.

Division of the Cytosome

The writer has observed *in both the diploid and the triploid* that during late anaphase and telophase of the first division a dark staining partition is formed in the equatorial region of the spindle fibers and at right angles to the longest axis of the fibers. (See diagrams of first division in figs. 31–37.) Occasionally pollen mother-cells (figs. 27–30) were seen in which there was no division of the cytosome. At the telophase of second division a partition is formed in the equatorial region of each spindle perpendicular to the longest axis of the spindle as well as perpendicular to the partition formed at the end of first division (fig. 48).

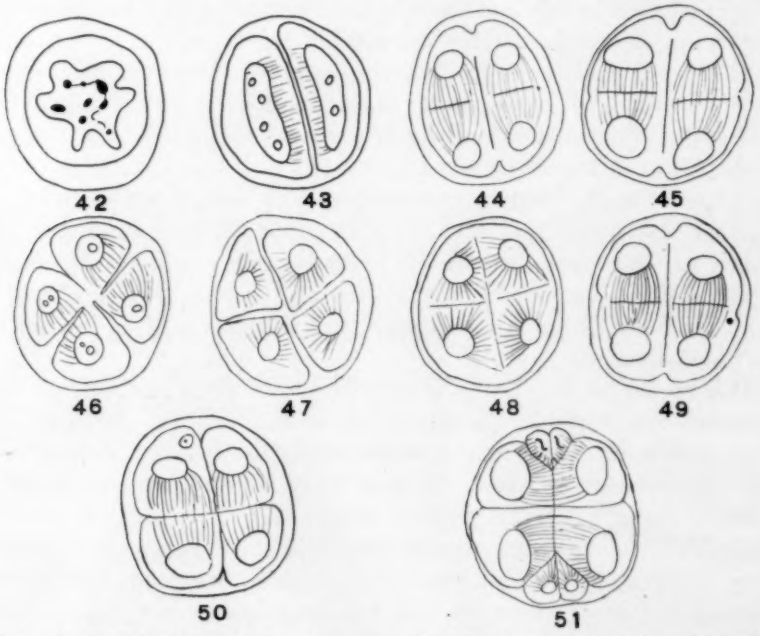
Furrowing begins at the periphery of the cytoplasm and proceeds inward along the region of the partition formed during first telophase (fig. 44). Shortly thereafter (almost simultaneously) a furrow begins at the periphery of the cytoplasm and proceeds inward along the region of the partitions formed during second telophase (fig. 45). These furrows continue until the microspores are completely separated (figs. 46, 47). The mode of division of the cytosome is therefore a simultaneous one. Strasburger (1882) thought that in *Hemerocallis fulva* the cytosome divides as a result of successive cell plate formations, while Tangl reported that the pollen mother-cell divides into four cells simultaneously. Yamaha (1926) reported that this division in *H. fulva* may be either successive or simultaneous.

In the triploid a few pollen mother-cells have been observed in which no division of the chromatic or the cytoplasmic contents had occurred. A single

nucleus, irregular in outline, persists (fig. 42). The chromatin material appears clumped, and each clump seems homogenous throughout as though some process of disintegration of chromatin material were well under way.

Sometimes only two cells are formed from a pollen mother-cell as reported by Stout and Susa (1929). The chromosomes in these nuclei have their chromatids widely separated except at the insertion region, which gives them the characteristic appearance of an X, V, or W, depending upon the position of the insertion region. In these cells it seems evident that the homoeotypic division has not occurred (fig. 43). Small microcytes may occur in addition to the two large cells. In such cases more than two cells may be formed at the heterotypic division and these cells may not undergo any further division.

The lagging units of chromatin for the most part are oriented on small spindles which are quite distinct from the two larger spindles of the cell.



FIGS. 42–51. Division of the cytosome. FIG. 42. Amoeboid nucleus in which no division of chromatin occurred. FIG. 43. Two cells resulting from first division in which the homoeotypic division has not occurred. FIG. 44. Furrowing first begins in the plane of the partition formed at first division. FIG. 45. Furrowing proceeds simultaneously in the plane of the partition found at second division. FIG. 46. Furrowing proceeds from the periphery toward the center of the pollen mother-cell until the spores are completely separated as seen in fig. 47. FIG. 48. All four nuclei connected by spindle fibers. FIG. 49. Microcyst present in the cytoplasm at the end of second division. FIG. 50. Microcyte in the cytoplasm of the cell at the end of second division. FIG. 51. Through simultaneous furrowing microcytes are formed which result in very small microspores.

Partitions are formed in the equatorial region of the secondary spindles perpendicular to their longest axis. Through simultaneous furrowing small microspores are formed (fig. 51). Frequently microcytes or microcysts (figs. 49–50) remain in the cytoplasm of the microspore and have been observed in the mature pollen grains.

THE NUMBER OF MICROSPORES

For the diploid it is the rule that four microspores result from two regular divisions of a pollen mother-cell.

In triploid plants from one to twenty microspores have been seen within a single pollen mother-cell wall. The irregular number of microspores as well as their unequal size can be explained by the manner of chromosome distribution at both the first and the second divisions. The principal irregularities at this time are as follows (see table 6):

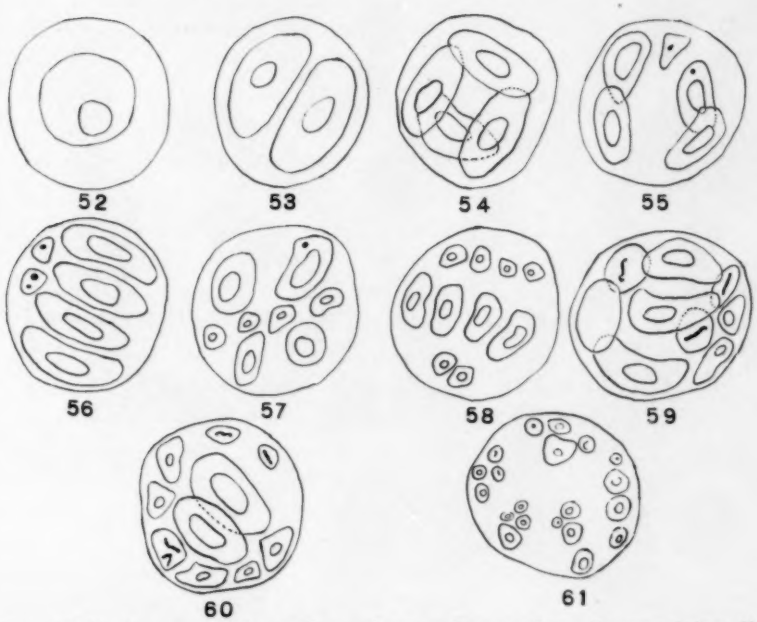
(1) Only two pollen mother cells have been seen in which there was no organization or division of chromatin (fig. 52).

(2) Two large cells within a single pollen mother-cell wall have been seen at the time when all surrounding pollen mother-cells have completed the homoeotypic division. Evidently only the heterotypic division occurred in these pollen mother-cells (fig. 53).

(3) Occasionally two large microspores with several smaller microspores have been observed within a single pollen mother-cell wall. From the appearance of the chromatin material in their nuclei, it is evident that they are the result of the heterotypic division and that no further division occurred. As many as ten microspores of various sizes may be formed at the end of first division.

(4) Frequently four large microspores with from one to four smaller microspores occur within a single pollen mother-cell wall (figs. 55, 56). It is clear from studies of second division metaphase that laggards of the first division may divide at second division. Four large spores may result from the two divisions of a pollen mother-cell (fig. 54).

(5) The smaller the microspores the fewer chromosomes there are in the nucleus of the spore. In cells where no cytoplasm is cut out and no nuclear membrane is formed about the lagging chromosomes, they round up, lose their characteristic shapes, and form microcysts in the cytoplasm of the microspore (figs. 57, 61). A large number of microspores is due to the formation of more than two nuclei (as many as ten have been observed) with the division of many or all of these in the homoeotypic stage. As many as 20 small microspores were observed within a single pollen mother-cell wall (fig. 61). Occasionally four large and five or six small microspores (figs. 58, 59) or two large and eight smaller microspores were seen within a pollen mother-cell wall (fig. 60).



FIGS. 52–61. Camera lucida outlines of ten pollen mother-cells containing different numbers of microspores. FIG. 52. Pollen mother-cell in which no division of nuclear material occurred. FIG. 53. Two cells which resulted from a single heterotypic division. FIG. 54. Four spores of nearly equal size. FIG. 55. Small cell containing microcyst but no nuclear organization, formed in addition to four cells of equal size, one of which contains a microcyst in its cytoplasm. FIGS. 56–61. Increased numbers of cells from single pollen mother-cells; the majority have organized nuclei, others have microcysts in the cytoplasm or a single chromosome which has not been dispersed.

Four spores of approximately the same size were observed in only 26.6 per cent of the pollen mother-cells studied.

TABLE 6

Number and relative sizes of spores formed from single pollen mother-cells

Spores from one mother-cell	Frequency
1 large spore	2
2 large spores	2
2 " " +2 small spores	1
2 " " +8 " "	1
3 " " +2 " "	4
3 " " +4 " "	1
4 large spores	29
4 " " +1 small spore	22
4 " " +2 " spores	25
4 " " +3 " "	8
4 " " +4 " "	9
4 " " +5 " "	1
4 " " +6 " "	1
4 " " +1 " " +2 smaller	1
5 spores all about the same size	1
20 small spores	1

THE MICROSPORE NUCLEUS AND ITS DIVISION

From the present study of the microspore of the diploid it is clear that a single, large, spherical nucleus is present in each microspore. In only a few spores has this nucleus appeared to be irregular in shape.

In the triploid the microspores which appear most normal usually have one large spherical nucleus. In it thin chromatin threads form a fine network. However, in pollen grains of similar size the nucleus appears to be oblong, oval, crescent-shaped, or amoeboid in outline; such grains may not be viable. A single large nucleolus is usually present in each nucleus, although as many as five nucleoli have been seen in a single nucleus. A somewhat thickened wall is developed about each microspore. In case of further development the nucleus moves to the periphery of the cell, where mitosis and cell division produce a large vegetative cell and a smaller generative cell. The latter soon migrates into the cytoplasm of the vegetative cell. This is completed at the time when the anthers are about four-fifths of their mature size.

The chromosomes in the early prophase of the mitosis of the microspore nucleus are long and comparatively straight. By the time they reach the equatorial plate they have become shortened to approximately one half the length seen in the earlier prophases. They are therefore compact and take stain very readily. In certain cells all chromosomes have been identified. The number of chromosomes participating in this division varies in different cells. The following numbers have been observed: 11 in one cell, 13 in one cell; 14 in four cells; 15 in two cells, 17 in two cells; and 18 in one cell. It is probable that further study would reveal a much lower number of chromosomes at the equatorial plate in mitosis of the vegetative nucleus, as reported by Stout and Susa (1929), who saw as few as six, or it might reveal as many as 22, which the breeding data suggest. It may be concluded that spores are formed which have other than eleven chromosomes.

MITOSIS OF THE GENERATIVE NUCLEUS

The generative cell divides to form two male gametes. This division may occur in the pollen grain before the pollen tube is formed or it may take place in the growing pollen tube.

The mitosis of the generative nucleus was studied in pollen tubes grown on artificial media. These preparations were prepared for study by the method described by Beatty (1937).

The pollen tube begins to protrude from the germinal aperture only a few minutes after pollen is scattered on the agar. Approximately two hours later the generative cell passes into the tube, and at the end of four hours early and late prophases, metaphases, and anaphases can be studied. In the diploid 11 chromosomes were frequently recognized at each pole of the anaphase (fig. 20).

In the triploid, mitosis of the generative nucleus was studied in the grain (fig. 22) as well as in the tube (fig. 21). In only a few tubes was the number of chromosomes determined for the generative nucleus. In one figure thirteen chromosomes were counted and identified. Presumably the variation in number would correspond to those in the vegetative cell.

VIABILITY OF THE POLLEN

It has been reported (Stout and Susa 1929) that germination of no more than 5 per cent was obtained for pollen of the triploid *H. fulva* clone Europa. Further tests of germination have given results in agreement with this earlier report. Only a few of the larger grains germinate (fig. 26). Many of the grains which do not germinate have protoplasmic contents. The pollen grains of the triploid are quite variable in size (fig. 24).

In the germination tests of pollen from the diploid at least 75 per cent of the grains produce long tubes (fig. 25). All the pollen grains have protoplasmic contents and appear to be quite uniform in size (fig. 23). Possibly the grains which did not germinate in these tests would do so with another treatment.

ABILITY OF POLLEN TO FUNCTION IN FERTILIZATION

There is much self- and cross-incompatibility in plants of *H. fulva*, but there has been no difficulty in obtaining seeds of diploid plants in pollinations that result in compatible fertilizations.

Twenty-three seedlings which have *H. fulva* clone Europa as one parent, either the seed parent or the pollen parent, and diploid daylilies for the other parent, have been grown at the New York Botanical Garden. The somatic numbers of chromosomes in these 23 plants ranged from 20 to 33. It seems evident (Stout 1932) that the gametes of the triploid which function most frequently in fertilization have 11 chromosomes, but that the number of chromosomes in functioning gametes may vary and rise to at least 22. The mechanism for the distribution of two sets of chromosomes into two equal groups or genoms does not seem adequate for the distribution of three sets into equal groups. Yet it does operate to the extent that some functional gametes are formed.

SUMMARY

1. The chromosomes have been identified in both diploid and triploid plants of *Hemerocallis fulva*. Two complete genoms of 11 chromosomes each were found in diploid plants grown from seed collected in China. Three such genoms were found in plants of the widely cultivated clone *H. fulva* clone Europa.

2. Meiosis proceeds regularly in the diploid plants and results in pollen grains of nearly uniform size, at least 75 per cent of which are viable when tested on artificial media.

3. The following irregularities have been found during meiosis in triploid plants:

a. The *associations* of homologous chromosomes in the early prophases are of four types. All three homologs may be rather closely associated and in such cases few chromomeres fail to become associated with other chromomeres. Close pairing of two of the homologs with a loose association of the third, which is the type described for the majority of triploids studied by various investigators, is frequent. A third chromosome may remain entirely unassociated. All three homologs of any one chromosome type may remain unassociated. These types of association result in the formation of univalents, bivalents, and trivalents.

b. The *distributions* of the chromosomes in the two divisions proceed with much irregularity and different numbers of chromosomes are distributed to the daughter nuclei. Lagging chromosomes were observed in the equatorial region at first and second anaphases. These later disintegrate in the cytoplasm, become vacuolated and dispersed in the vacuoles of the cytoplasm, thus forming microcysts, or become organized in small nuclei included in microcytes.

c. *Irregular numbers of nuclei* are formed at both the first and the second divisions as a result of the incomplete association of homologous chromosomes in the prophases, and of the unequal distribution of chromosomes during the first and second anaphases.

d. *Irregular numbers of microspores* are formed from a single pollen mother-cell. These vary in size, depending upon the number of chromosomes contained in their nuclei. In only 25 per cent of the pollen mother-cells studied were four spores formed that were nearly equal in size. As many as 20 small cells may be formed from one pollen mother-cell.

4. No more than five per cent of the mature pollen grains of this triploid clone germinate on artificial media. Owing to self-incompatibility no seeds have been obtained from this clone to self- or close-pollinations, but capsules are formed from certain hybridizing pollinations with other species and as many as five seeds have been obtained in one capsule. This low number of seeds in ovaries which contain as many as 30 ovules is evidence of abortion during megasporogensis as during microsporogenesis.

5. The chromosomes of the diploid and of the triploid are remarkably alike in form, size, and shape. No evidence of structural hybridity was observed. It is evident that this triploid clone, Europa, arose from a diploid plant by autopolyploidy.

6. It is the general if not the universal rule that triploid plants exhibit much sterility due to abortion of spores. This is the condition even in autotriploids. In the Europa daylily, at least, the irregularities leading to abortions seem to be due to the third set of chromosomes and the effect which its members have on the mechanism of distribution.

The writer feels deeply indebted to Dr. A. B. Stout for his stimulating criticism and valuable suggestions during the progress of this study and in the preparation of this paper.

THE NEW YORK BOTANICAL GARDEN
NEW YORK, N. Y.

Literature Cited

Beatty, A. V. 1937. A method for growing and for making permanent slides of pollen tubes. Stain Tech. **12**: 13–14.

Belling, J. 1921. Counting chromosomes in pollen-mother cells. Am. Nat. **55**: 573–574.

———. 1925. Chromosomes of *Canna* and of *Hemerocallis*. Jour. Hered. **16**: 465–466.

———. 1929. Nodes and internodes of trivalents of *Hyacinthus*. Univ. Calif. Publ. Bot. **14**: 379–388.

Biourge, P. 1892. Recherches morphologiques et chemiques sur les grains de pollen. La Cellule **8**: 47–76.

Chandler, C., Porterfield, W. M. & Stout, A. B. 1937. Microsporogenesis in diploid and triploid types of *Lilium tigrinum* with special references to abortions. Cytologia **Fujii Jub.**: 756–784.

Dark, S. O. S. 1932. Meiosis in diploid and triploid *Hemerocallis*. New Phytol. **31**: 310–320.

Eisen, G. 1899. The chromoplasts and the chromioles. Biol. Centralbl. **19**: 130–136.

Fullmer, E. L. 1899. The development of the microsporangia and microspores of *Hemerocallis fulva*. Bot. Gaz. **28**: 81–88.

Heitz, E. 1926. Der Nachweis der Chromosomen. Vergleichende Studien über ihre Zahl, Grösse und Form im Pflanzenreich I. Zeits. Bot. **18**: 625–679.

Horton, E. S. 1936. Studies in the cytology of wheat species hybrid. Am. Jour. Bot. **23**: 121–129.

Juel, H. O. 1897. Die Kernteilungen in den Pollenmutterzellen von *Hemerocallis fulva* und die bei denselben auftretenden Unregelmässigkeiten. Jahrb. Wiss. Bot. **30**: 205–226.

Lobel, M. 1576. Plantarum seu stirpium historia.

McClintock, B. 1929. A method for making aceto-carmin smears permanent. Stain Tech. **4**: 53–56.

Matsuura, M. & Sutô, T. 1935. Contributions to the idiogram study in phanerogamous plants. I. Jour. Fac. Sci. Hokkaido Imp. Univ. V **5**: 1–75.

Olmo, H. P. 1934. Prophase association in triploid *Nicotiana tabacum*. Cytologia **5**: 417–431.

Pfitzner, W. 1881. Morph. Jahrb. **7**: 289–311.

Schürhoff, P. N. 1913. Karyomerenbildung in den Pollenkörnern von *Hemerocallis fulva*. Jahrb. Wiss. Bot. **52**: 405–409.

Sienicka, A. 1929. Ueber den Blütenbau und die Pollenbildungsvorgänge bei *Hemerocallis fulva fl. ploeno*. Acta Bot. Polon. **6**: 296–334.

Skovsted, A. 1933. Cytological studies in cotton. I. The mitosis and the meiosis in diploid and triploid Asiatic cotton. Ann. Bot. **47**: 227–251.

Stout, A. B. 1932. Chromosome numbers in *Hemerocallis*, with reference to triploidy and secondary polyploidy. Cytologia **3**: 250–259.

———. 1934. Daylilies. Macmillan.

Stout, A. B. & Chandler, C. 1933. Pollen-tube behavior in *Hemerocallis* with special reference to incompatibilities. Bull. Torrey Club. **60**: 397–417.

Stout, A. B. & Susa, T. 1929. Chromosome irregularities in relation to sterility in *Hemerocallis fulva* elon **Europa.** Ann. N. Y. Acad. Sci. **31:** 1–30.

Strasburger, E. 1882. Ueber den Teilungsvorgang der Zellkerne und das Verhältnis der Kernteilung zur Zellteilung. Arch. Mikr. Anat. **21:** 476–590.

Takenaka, Y. 1929. Karyological studies in *Hemerocallis.* Cytologia **1:** 76–84.

Tangl, E. 1882. Die Kern-und Zelltheilungen bei der Bildung des Pollens von *Hemerocallis fulva* L. Denks. Akad. Wien. **45:** 65–84.

Timm, H. 1928. Untersuchungen über die Sterilitätsursachen von *Hemerocallis fulva* und *citrina.* Planta **5:** 784–808.

Tischler, G. 1915. Chromosomenzahl, -Form und-Individualität im Pflanzenreiche. Prog. Rei Bot. **5:** 164–284.

Warmke, H. E. 1935. A permanent root tip smear method. Stain Tech. **10:** 101–103.

Wodehouse, R. P. 1935. Pollen grains. McGraw-Hill.

Yamaha, G. 1926. Ueber die Zytokinese bei der Pollentetradenbildung, zugleich weitere Beiträge zur Kenntnis über die zytokinese im Pflanzenreihe. Jap. Jour. Bot. **3:** 139–162.

INFECTION STUDIES ON THE COVERED SMUT OF OATS[1, 2]

PAUL F. BRANDWEIN

Infection in the oat plant has been thought to be the result of a close relationship between the early development of the oat and the development of the smut, the environment playing a significant role. An investigation to determine the effective period of infection in the oat seedling seemed desirable and is here set forth.

This work is possible only through a technique by which 100 per cent infection of a susceptible variety was obtainable. Reed and Faris (25) present a method which involves dry spore inoculation of seeds whose hulls have been removed. These seeds are germinated under constant environmental conditions, optimum for infection. The discovery by Reed (19) that there are among the oat smuts certain physiologic races which differ in their ability to infect a selected oat variety made it possible to inoculate seed with smut of a known race.

REVIEW OF PREVIOUS WORK

In this paper it is not necessary to review the controversy whether the flower or the seedling is the original infection site. An excellent review of this question is offered by Kolk (14). It suffices to state that while several investigators[3] have offered evidence that flower infection occurs, it is equally evident from the work of others[4] that high infection percentages occur when the plant is infected by dry or germinated spores placed on the seed or seedling.

Some evidence is available to show that the young oat seedling may be infected, but not after the first leaf breaks through the coleoptile. Brefeld (5) described typical penetration holes in a three-day-old oat seedling and determined roughly the period of infection. He obtained 17–20 per cent infection of seedlings 2.5 mm. long, 7–10 per cent infection of seedlings 1 cm. long, and 2 per cent infection of seedlings 1.5–2 cm. long. Seedlings whose coleoptiles were penetrated by the first leaf yielded 0–1 per cent infection, whereas inoculation of older plant tissues and the growing point failed. He concluded, therefore, that the younger the seedling the more susceptible it is to infection.

Kolk (14) studied penetration and development of *Ustilago Avenae*. She states that the germ tubes from conidia may penetrate the oat coleoptile

[1] Brooklyn Botanic Garden Contribution No. 91.

[2] Accepted in partial fulfillment of the degree of Doctor of Philosophy at New York University, October 21, 1939.

[3] Brefeld and Falk (6), Zade (34), Arland (1), Diehl (7), Roesch (27) working with *Ustilago Avenae* in Germany, and Gage (9) in the United States.

[4] Kühn (15), Wolff (33), Brefeld (5), Reed and Faris (25), von Rosenstiel (28), Nicolaisen (18), and Kolk (14).

at any place from its tip to its node. She did not find mycelium in the scutellum, the root node, or the lower portion of the mesocotyl of 1–4-day-old seedlings. In seedlings 5–8 days old the mycelium was distributed in the tissues of the coleoptile and mesocotyl. Kolk also found mycelium in the coleoptile of Black Mesdag, a variety resistant to the smut she was studying. Since then, Western (32) and Brandwein (3) have found considerable evidence from a number of oat varieties that the smut fungus may penetrate the coleoptile of resistant seedlings. Roesch (27) inoculated seedlings varying from 3 mm. to 3 cm. in length. He states that the infection period of the young oat seedling disappears with the eruption of the first leaf.

Studies on the physiology and ecology of infection augment the evidence obtained through histological investigation. McAlpine (17) suggested that whatever hinders the rapid development of the seedling and extends the periods of infection favors the access of the fungus to the growing point. Hiltner (10), however, maintains that while the highly susceptible Fichtelgebirg's oats penetrate the soil as rapidly as the resistant Ligowa variety, the former is markedly retarded in later development. This, according to Hiltner, may explain the greater susceptibility of Fichtelgebirg's oats. Reed and Faris (25) conclude that under controlled conditions of moisture, temperature, and soil reaction, the greater susceptibility to *Ustilago levis* of *Avena nuda* as compared with the Victor oat cannot be explained by differences in the rate of germination of the seeds. These authors, like McAlpine, raise the question of the apparent failure of very susceptible plants to give infection percentages of 100 per cent. They suggest that the explanation may be in the difference in the rate of growth of individual seedlings.

Since then Reed (24) has presented excellent evidence that infection of oats occurs in the seedling and that variation of nutrition and light in the post-seedling stage has no effect on infection. The evidence indicates that the first four days in the life of the plant are important and that treatment unfavorable to rapid growth of the plant in the post-seedling stage does not increase infection. Tapke (31), however, reports that environmental conditions after seedlings emerge may markedly influence the incidence of smut. He points out that the seedling-infecting smuts such as the loose smut of oats and the covered smut of barley invade their hosts during emergence of the seedling from the soil. Under "pre-emergence conditions" favorable for this smut invasion similar percentages of smut occurred in both the outdoor and greenhouse environments, while under "unfavorable pre-emergence conditions" greenhouse plants had a higher incidence of smut than those grown in the field. Tapke concludes that "the rugged outdoor environment sustained and the temperate greenhouse conditions ameliorated the effects of the relatively unfavorable pre-emergence conditions." A proper evaluation of these results must await more complete data, since it must be based on a com-

plete knowledge of what is meant by "favorable" and "unfavorable pre-emergence conditions."

On the basis of the data reported here, it seems safe to predict that an effective period of infection should be found in the oat seedling. The determination of such a period is itself of considerable interest and value in view of the various suggestions found in the literature. At the same time, some further knowledge may be gleaned about the structural or physiological modifications which the host undergoes as it passes from extreme susceptibility to a degree of immunity in the mature plant. It may be that this immunity of the mature susceptible plant is not physiological immunity of the growing point but is due to morphological changes involved in maturation.

Before proceeding further, it is necessary to establish a criterion of infection. In the normal relation between host and parasite, the smut fungus penetrates the oat seedling, establishes itself in the growing point, and forms chlamydospores at maturity. From the foregoing summary of the literature, it appears that the fungus may enter the oat seedling but may not sporulate. Yet it is clear that the latter condition is as much an infection as the former. For the purpose of description, infection of the oat may be defined as a relationship which terminates with the sporulation of the fungus. Any mention of infection in this paper should be interpreted in this way. However, opportunity will be taken in the discussion for a clarification of this question.

EXPERIMENTAL METHOD

Three varieties of *Avena sativa* L. were used in these experiments, Monarch (S.N. 161), Black Mesdag (S.N. 70), and Markton (S.N. 752). The smut used was *Ustilago levis* race 7, to which Monarch is 100 per cent susceptible, Black Mesdag partially susceptible, and Markton resistant. The smut, as well as the oat varieties, are propagated by Dr. G. M. Reed at the Brooklyn Botanic Garden.

The technique used in the inoculation, germination, and subsequent planting of the oats was the same as that previously described by the writer (3). For the purposes of this investigation, however, all seeds under 8 mm. in size were rejected. The seeds were germinated, groove downward, in sand contained in paraffined paper cups. The sand was moistened to 20 per cent of its water-holding capacity and the cups were placed in an incubator maintained at 20° C.

THE EFFECTIVE PERIOD OF INFECTION

To determine the effective period of infection, lots of seeds and seedlings were inoculated every six hours with dry chlamydospores. After the plants were generously dusted with spores from a rubber bulb, fresh sand of the same moisture content was carefully placed around and over them. Most of

the seedlings emerged from the sand 96–98[5] hours after planting; 12–16 hours later, rarely earlier, the first leaf broke through the coleoptile.

A summary of the results obtained is presented in table 1. The percentages of infection are the averages of the results of four plantings, two in the greenhouse in 1938 and 1939 and two in the field in 1937 and 1939.

TABLE 1

Infection of oat varieties inoculated with Ustilago levis race 7, at different hours

Hour inoculated	Probable period of infection	Monarch		Black Mesdag		Markton	
(from date of planting)	factor[1] 36–42 hours + inoculation time (column 1)	Number of plants	Percentage infection	Number of plants	Percentage infection	Number of plants	Percentage infection
0	36–42	110	100.0	110	79.0	110	2.7
6	42–48	110	99.1	110	85.5	108	0
12	48–54	108	100.0	106	61.3	105	0
18	54–60	106	100.0	107	46.7	102	1.9
24	60–66	110	100.0	105	40.9	110	0
30	66–72	106	98.1	104	46.6	109	0
36	72–78	104	91.4	100	37.0	108	0
42	78–84	106	84.9	109	27.5	107	0
48	84–90	105	86.7	106	32.1	110	0
54	90–96	110	63.6	99	19.1	97	0
60	96–102	104	56.7	103	8.7	104	0
66	102–108	101	69.3	108	0.0	102	0
72	108–114	106	33.0	100	1.0	89	0
78	114–120	100	28.0	100	3.0	101	0
84	120–126	98	10.2	91	0.0	88	0
90	126–132	97	5.2	102	0.0	101	0
96	132–138	102	4.9	106	0.0	100	0
102	138–144	94	2.1	90	2.2	99	0
108	144–150	104	0.0	103	0.0	107	0
114	150–156	105	0.0	102	0.0	105	0
120	156–162	108	0.0	98	0.0	110	0
126	162–168	106	1.9	110	0.0	110	0
132	168–174	105	0.0	102	0.0	108	0
138	174–180	107	0.0	103	0.0	109	0
144	180–186	108	0.0	101	0.0	107	0

[1] 36–42 hours is the approximate period of actual infection determined (table 2).

It was not thought desirable to carry out inoculation experiments with germinated spores, with conidia, or with smut mycelium, since the introduction of the inoculum would entail the addition of the medium in which it was grown.

Table 1 clearly demonstrates that the effective period of infection of susceptible and partially susceptible varieties occurs during the earliest stages of development. It further demonstrates that, as the seedlings develop, their

[5] The age of all the seedlings is calculated from the time at which the seeds were placed in sand.

capacity for infection is correspondingly limited. After the first leaf has emerged the capacity for infection is zero or nearly so.

A comparison of the three varieties is interesting. Markton, which is highly resistant, showed 2.7 per cent and 1.9 per cent infection respectively when inoculated at the zero and 18-hour stages, but was completely resistant thereafter. Black Mesdag, which is partly susceptible to *U. levis* race 7, gave infection percentages of 1, 3, and 2.2 after 66 hours, but 70.9 per cent and 85.5 per cent at zero and 6 hours, with figures decreasing to 8.7 per cent at 60 hours. On the other hand, Monarch showed 100 per cent infection (with some slight variations) through the 0–30-hour inoculation period, with percentages of infection decreasing to 4.9 per cent at 96 hours. After this period, subsequent inoculation produced 2.1 per cent infection at 102 hours and 1.9 per cent at 126 hours after planting.

Determination of the Actual Period of Infection

The data in table 1 become more significant when the actual time of infection is established. Since dusting of the seed with the chlamydospore is the method of inoculation used by many workers, this method was employed.

Preliminary experiments established that inoculation of seedlings exposed to air failed to cause infection. Since such exposure did not injure the seedlings it was possible to perform the following experiment. Seedlings 24 hours old were dusted with spores, covered with properly moistened inoculated sand, and placed at 20° C. At intervals of 20, 24, 28, 32, 36, 40, and 44 hours the cups were removed from the incubator, the surface sand removed, and the seedlings exposed to air. The cups were watered from below. Seedlings of Black Mesdag were similarly treated. The seedlings were transplanted to soil in pots from four to eight days after the emergence of the first leaf and grown to maturity.

Table 2 illustrates that for the Monarch oat, 40–44 hours contact with

TABLE 2

Infection time of oat seedlings inoculated with Ustilago levis race 7[1]

Time of exposure (elapsed hours)[2]	20	24	28	32	36	40	44
Percentage infection Black Mesdag	10.5	40	48.6	43.9	50	62.1	55
Percentage infection Monarch	15	27.2	59.8	90	91.4	100	100

[1] 40 seedlings were used in each determination, percentages being based on number of surviving plants.

[2] The 24 hour seedlings were all inoculated at the same time. "Time of exposure" indicates number of hours elapsed when the surface layer of sand was removed and the seedling exposed to air to stop infection; i.e., 24 hours added to "Time of Exposure" = age of seedling.

spores in sand resulted in 100 per cent infection. Twenty hours of exposure to the spores produced 10.5 per cent infection. This is in accordance with observations (Jones 16) that the dry spores may germinate within 6 to 24 hours under conditions suitable for germination. At the latter time, maximum germination appears to have occurred. Black Mesdag gave variable results; maximum infection appears to have occurred after 40 hours contact with spores in sand. Perhaps the infection percentage at 40 hours was maximum for that group of seedlings since, at best, infection percentages for Black Mesdag may vary from 50 to 85 per cent. It seems safe to consider from 36 to 42 hours as the period necessary for maximum infection of Monarch and Black Mesdag seedlings. It is conceivable, however, that seedlings in the later stages of maturity might require a longer period of contact with spores for infection to take place.

If table 1 is now examined more closely, it may be seen that infection of Monarch may be considered to occur during the period between 36 and 78 hours after planting in sand. This infection period is obtained by adding 36–42 hours to the inoculation period (column 2, table 1). During this 36–78-hour period, infection percentages of 100–91 per cent are obtained. After 78 hours from the time of planting, the infection percentages fall rapidly. Seedlings inoculated at 72 hours after planting were probably infected during a period of 20–40 hours (or slightly longer) after the inoculation (table 2), that is, at 92–112 hours after planting; while Monarch seedlings inoculated after 90 hours in sand were probably infected at 110–130 hours after planting. The latter period occurs after the emergence of the first leaf.

The figures for infection of Black Mesdag (table 1) are variable; the highest are those at the inoculation times of 0, 6, and 12 hours. At 30 hours after planting, there is a percentage of 46.6 infection, as compared with 61.3 per cent at 12 hours after planting.

The lower limit of the period of maximum infection for Black Mesdag occurs at about the same time as that for Monarch. Since Black Mesdag is only partially susceptible, a determination of a more exact limit of maximum infection time would have little value.

The seedlings most favorable for the study of penetration were 48–72 hours old, the evidence of penetration being very plentiful. Yet promycelia and conidia with fusion hyphae appeared to be as numerous during the 36-hour stage after planting as at the 72-hour stage.

It was necessary to ascertain whether seedlings could be infected during the first 36 hours. It had previously been found by Reed and Faris (25) that seedlings of Victor oats were not infected when they were germinated at 5° C. with 40 per cent moisture. It was thus possible to stop infection of Victor oats; possibly this could be applied to Monarch as well.

Two series of plantings were made. In one series, the seeds, planted in sand moistened to 20 per cent of its water holding capacity and maintained at 20° C., were inoculated with chlamydospores; in another, the seeds, planted in the same manner, were inoculated with spores germinated in a 2 per cent sucrose solution for 24 hours at room temperature. The latter bore abundant promycelia, conidia, and fusion hyphae. The solution containing germinating spores was applied with a pipette; accordingly the moisture content of these seedling cultures is somewhat higher. At six-hour intervals, the cups were removed from the incubator and the seedlings were transplanted in sand moistened to 40 per cent of its water-holding capacity and placed at 5° C. In one month most of the seedlings had penetrated the sand. In the inoculated control grown in sand at 40 per cent moisture and 5° C. one plant was infected out of a total of 36.

The results showed that up to 24 hours at 20° C. and 20 per cent moisture the infection by dry spores was zero, but with the germinated promycelia it was 8.4 per cent. After 42 hours maximum infection was reached, while after 36 hours 90.9 per cent infection was obtained with germinated spores. Nevertheless, we must question the accuracy of the results obtained with the germinated spores since numerous bacteria were found in all the cultures. On the other hand, plants inoculated with the dry chlamydospores were 72.5 per cent infected after 36 hours and 96.8 per cent infected after 42 hours at 20° C. and 20 per cent moisture. It seems that 100 per cent infection is uncertain before 42 hours when the seed is inoculated with the dry spores.

In the light of these experiments and suggestions in the literature, four stages may be suggested in the effective period of infection of the highly susceptible seedling Monarch. It must be emphasized that this refers only to conditions in which maximum infections of 100 per cent may be realized. These are as follows: (1) Lower limit of moderate infection; from 18 to approximately 36 hours after planting. (2) Period of maximum infection; from 36 to approximately 72 hours after planting. (3) Higher limit of moderate infection; from 72 hours to approximately 132 hours after planting. (4) Period of non-infection. This refers to stages past 132 hours in which inoculation of the normal plant with dry spores has been completely ineffective. A modification of this last point will be presented later.

A summary of the data from tables 1 and 2 shows that Black Mesdag, a form moderately susceptible to *Ustilago levis* race 7, has an effective period of infection limited to the first 100 hours, under the environmental conditions maintained. Of these 100 hours, the period between 36 and 54 hours after planting probably constitutes the effective period of maximum infection. On the other hand, Monarch, a completely susceptible variety, has an effective period of infection of approximately 132 hours. Of these 132 hours,

the period between 36 and 72 hours after planting probably constitutes the period of maximum infection. It is apparent that the susceptible variety has not only a longer effective period of infection but a longer effective period of maximum infection.

Correlation of Growth Rate and Maturation with Effective Period of Infection

Growth measurements of the three varieties, Monarch, Black Mesdag, and Markton, were made to determine whether their growth rate differed. All seedlings were grown under identical conditions at 20° C. in coarse sand of 20 per cent moisture content. Forty seedlings were removed from the sand every 12 hours over a four-day period and measured.

A comparison of the figures demonstrated that the growth rate of all was remarkably similar at every period. In fact all the seedlings germinated and penetrated the sand at approximately the same time. It is surprising that Monarch, the most susceptible of them all, grew slightly more rapidly than Black Mesdag or Markton. Obviously the greater susceptibility of Monarch cannot be explained on the basis of a difference in germination and growth rate. The foregoing experiments, however, throw some light on the nature of infection. It is seen that the highly susceptible Monarch, the moderately susceptible Black Mesdag, and the resistant Markton grow at approximately the same rate. It is equally clear that the effective period of infection for Black Mesdag is shorter than that of Monarch. It seems, then, that development in size masks a development of resistance to infection; the latter, however, is not correlated with development in size.

It is necessary to explain, however, why susceptible plants sometimes vary markedly in percentage of infection. McAlpine (17) tried to explain unequal infection on the assumption that the host plant must be at the right stage of development when the spore or conidium has put forth its germ tube and that this period does not last long.

In this paper evidence has been presented that a short period of infection does exist for the partly susceptible Black Mesdag. Furthermore, experience with growth measurements in seedlings of Black Mesdag shows the great variability in development at any one stage; this is also true of Monarch and Markton. Especially is this true of the difference in mesocotyl and coleoptile length of different seedlings. At 84 hours after planting, this variability is even more noticeable than it is at 72 hours. Beyer (2) also mentions the fact that this variability, especially of the mesocotyl length, has been a serious obstacle to all those who have attempted to study the physiology of the oat seedling.

THE REGION OF GREATEST INFECTION IN THE SEEDLING

McAlpine (17) remarks that unequal infection is the crux of the whole position; if we could account for it satisfactorily it would help us to under-

stand the conditions under which infection occurs. Two other types of experiments were planned, therefore, in the hope that the situation might be further clarified. One experiment was planned to determine the region of greatest infection in the seedling; another to determine whether or not it was possible to infect mature plants in the post-seedling stage, after the eruption of the first leaf. In this way it might be determined whether the immunity of the mature plant is due to a physiologic state or merely to the protection of the growing point by the outer tissues.

The method used to determine the regions of greatest infectibility was simple though tedious. For infection of the coleoptile tip the seedlings were surrounded by fine sand, with 1–2 mm. of the tips exposed to dusting with spores. For infection of the lower end of the shoot, about 2 mm. were exposed. Many methods were attempted; the most satisfactory was to place a hood of tinfoil over the seedling. Inoculations were made before planting and 48, 72, and 84 hours after. After inoculation, the hood was carefully removed and the seedling covered with coarse sand of the required moisture content. The seedlings were transplanted to pots in the greenhouse after the usual treatment.

The results are summarized in table 3. It is apparent that when different portions of the seed are inoculated, inoculation of the proximal end results

TABLE 3

Region of highest infection of Monarch oat seedlings[1]

Stage	Seed (at time of planting)		48 hours (after planting)		72 hours (after planting)		84 hours (after planting)	
Where inoculated	Proximal End	Distal End	Seed End	Coleoptile Tip	Seed End	Coleoptile Tip	Seed End	Coleoptile Tip
Percentage infection	100	15[2]	80[3]	20	2[4]	2[4]	0	2
Percentage infection Table 1 (Entire Plant)	100		85.7		33		10.2	

[1] About 2 mm. were exposed for inoculation; 50 seedlings were used for each regional inoculation.

[2] In these plants only the tillers were infected, not the main panicle.

[3] Five plants with tillers only infected.

[4] One plant with tillers only infected.

in the highest infection. The 15 per cent infections obtained by inoculating the distal end may be due to faulty technique. It is possible that several spores or the developing mycelium reached the proximal end after the removal of the tinfoil. Theoretically, only one germinating chlamydospore is

necessary for infection. After 48 hours from planting, inoculation of the seed end of the seedling caused infection of 80 per cent, while 20 per cent were infected by inoculation of the tip. Infection of 2 per cent was obtained by inoculation of both seed end and coleoptile end of seedlings 72 hours old. No infection was obtained when the coleoptile tip or seed end of seedlings 84 hours old was inoculated. These seedlings were studied more carefully by inoculating successive areas beginning with the seed end of the shoot. They were treated as before, except that an additional 2–3 mm. were exposed at each inoculation. This 20 mm. shoot was thus divided into 7 regions. The results are summarized in table 4.

TABLE 4

Regional inoculation of the Monarch seedling[1]

Regions	Number infected	Number of plants	Region exposed
Seed end	0	10	Mesocotyl
6 mm.	0	10	Mesocotyl
9 mm.	0	9	Mesocotyl near coleoptile node
12 mm.	0	10	Lowest part of coleoptile below growing point and mesocotyl
15 mm.	1	9	Coleoptile node and mesocotyl
18 mm.	1	10	Coleoptile and mesocotyl
20 mm.	2[2]	10	Coleoptile and mesocotyl

[1] All seedlings were inoculated 84 hours after planting.
[2] 1 plant with tillers only infected.

It is apparent that inoculation of the mesocotyl caused no infection. Yet histological examination of the epidermis of such seedlings shows abundant penetration by the fungus of both mesocotyl and coleoptile. Kühn (15) working with bunt, maintained that infection was possible only through the portion of the seedling between the root node and coleoptile node. His conclusions, he thought, were strengthened by his discovery of germ tubes in the tissues of the coleoptile of barley seedlings which grew into sound plants. Lang (16), working with loose smut of oats, thought that the mesocotyl was the point of entry. Kolk (14) found mycelium in the coleoptile and the mesocotyl of 73 oat seedlings inoculated with loose smut. The results reported here indicate, however, that penetration and subsequent establishment of the fungus in the plant tissue does not mean infection in the sense that the parasite will sporulate.

A study of seedling growth shows that 48 hours after planting the seedling is about 4.5 mm. long. This is about 17 per cent of the entire growth (averaging 28 mm.) of four days. After 72 hours the seedling is about 14 mm. long; in the next twenty-four hours the seedling grows an additional 14 mm. In the last two days, 83 per cent of the entire four-day growth has

occurred, an increase in rate of approximately 480 per cent over that of the first two days.

The period of maximum infection has been shown to exist during the first 72 hours after germination. For Monarch, this period may be correlated with the early slow growth rate. Similarly the period of decreasing infection begins at 48 hours after planting; it is to be correlated with the very rapid growth rate after this time.

With the help of this evidence, the story of infection in completely susceptible varieties may be reconstructed as follows: When the seed is inoculated and placed under suitable environmental conditions, the spores germinate and the fungus penetrates the plant between 36 and 42 hours after planting. The fungus flourishes within the plant and eventually is established in the growing point, where it remains until sporulation. Spores, however, vary in their time of germination and the fungus may penetrate the base of the plant 72 hours after planting. If it does it may remain in the mesocotyl, which rapidly elongates, carrying with it the growing point. If the fungus penetrates earlier (60 hours after planting) it may eventually reach the root node, where it may remain until tillers are produced and infect the growing points of these. On the other hand, penetration by the fungus at the base of the plant 84–96 hours after planting will probably result in its establishment in the tissues beneath the growing point. Sporulation may not occur. If it is established in mesocotyl and coleoptile only, it will die when these structures degenerate.

While the mycelium may grow as rapidly in mature tissue as in the immature seedling, it is certain that a hypha entering at the seed end of a seedling 84 hours old must traverse over 11 mm. to reach the growing point.[6] If the fungus enters 24–36 hours after planting, it is directly on the level of the growing point. Examination of hyphae in the coleoptile and mesocotyl of Monarch seedlings show that they ramify in these tissues and do not grow straight towards the growing point. It is highly probable, therefore, that the hyphae may ramify in the coleoptile or mesocotyl and go no further, or they may reach the crown node or the growing point.

It is also probable that the growth of the mycelium in the mature plant is slower than that in the young seedling. Measurements of cells of oat seedlings 24, 48, 72, and 96 hours after planting show a great increase in length of these cells as the seedlings mature. About 20 cells in approximately similar localities were measured on strips of epidermis of five seedlings. The average length follows:

24 hours—30.2 μ	72 hours— 76.2 μ
48 hours—48.5 μ	96 hours—194.2 μ

[6] This figure is derived from a measurement of the average distance of the growing point from the seed end: about 11 mm. at 84 hours after planting.

The width of the cells increased proportionately. The cell walls increase in thickness from about 2 μ (24 hours after planting) to about 4 or 5 μ (60 hours after planting). Kolk (14) has shown that the hyphae she was studying tended to remain in the peripheral cytoplasm of the oat cell and did not penetrate the vacuole. There is sufficient reason to assume, therefore, that the smut mycelium may be retarded in its growth toward the growing point by the thicker walls, the larger cells, and by the tonoplast. Altogether, the distance the hypha must traverse, and the obstacles present in its path toward the growing point, help to account for the failure to realize always 100 per cent or other high infection percentages in seedlings of the Monarch oat inoculated 48 hours after planting.

While low percentages of infection of a highly susceptible variety may be satisfactorily explained on the basis of an increased growth rate and on maturation phenomena such as thickening of the cell wall, appearance of vacuoles, and cell elongation in the late seedling, this explanation may not be used for moderately susceptible and resistant varieties such as Black Mesdag and Markton. These two grow at a rate remarkably similar to that of Monarch. In other words, these two varieties should, if growth and maturation are the only factors to consider in immunity to smut infection, be infected as easily as Monarch.

INFECTION OF MATURE PLANTS THROUGH INOCULATION OF THE GROWING POINT

It was thought that inoculation of the growing point of mature plants should produce infection. Faris and Reed (8) have shown that mature injured plants of sorghum may be infected. Injections into the growing point and surrounding tissue of germinated and dry chlamydospores in various media by hypodermic needle failed to produce results in 1936, 1937, and 1938. Finally, in 1939, oat plants were successfully grown to maturity in paraffined paper cups and the following experiments proved successful. Plants were grown for three weeks during the spring growing period and for six weeks in the fall growing period. After this, the bottoms of the cups were pierced and the cups placed in moist chambers containing an inch of water. The leaves of the plants were removed and each plant was slit medially to the growing point node with a sharp razor. For three successive days, the plants were covered with a fine spray of a 1 per cent sucrose solution, then dusted with the dry chlamydospores. After one week and a half the covers were removed from the moist chambers. The plants were about 1.5 feet in height at fruiting.

The results, as may well be expected, were erratic. In one series of 30 Monarch plants, four were infected; in another series of 25 plants, 24 were infected. In Black Mesdag, 5 plants out of 52 were infected, while no infection of Markton could be obtained. An improved inoculation technique may

yield greater percentages of infection. On the basis of these results more significance may be attached to the role of rate of growth and maturation in decreasing the infection of a completely susceptible variety such as Monarch. The rapid rate of growth and maturation of the seedling presents certain obstacles to the smut fungus in its attempts to reach the growing point, which, however, remains susceptible.

With the discovery that oat seedlings could be grown to maturity in the paraffined paper cups, the three varieties were grown in this manner to determine whether post-seedling stunting of growth would increase infection. The percentages of infection of stunted plants were compared with those obtained by the writer for normal plants and found to be very similar.[7] At the same time, Dr. Reed (24) was conducting experiments which demonstrated that stunting the post-seedling plant by varying nutrition and light did not affect the percentage of infection. This is definite evidence that in oats the infection phenomena during approximately the first five days of the life of the plant are important. Modifications of growth or nutrition thereafter do not alter, at least in any externally visible characteristic, the relations between host and parasite established during these first few days.

Finally, it has been thought that a non-sporulating infection may adversely affect the oat varieties in which such infection is present. Brandwein (3), working with controlled conditions and known races of smut, could not substantiate the report of Hubbard and Stanton (11) that such varieties were adversely affected in yield and height through inoculation with smut. Stevens (30) also was unable to demonstrate an adverse effect on mature Markton plants. The work here presented further confirms the writer's previous work. Of the plants inoculated at different hours (table 1) many which had been inoculated 60–96 hours after planting produced normal main heads and were counted as non-infected. In no way could these plants be distinguished from others in the row or in the plot. Yet from one to two weeks later many of these plants produced infected tillers. Measurements of the height of main stalks, number of culms, and yield of these plants, and a comparison with an equal number of normal plants chosen at random from the same rows showed little difference, as can be seen from the following:

	Monarch		*Black Mesdag*	
	Normal	*Infected Tillers*	*Normal*	*Infected Tillers*
Number of plants	31	31	23	23
Average height in cm.	71.2	73.9	80.7	78.7
Average number of culms	7.2	8.9	6.1	6.3
Weight of main head in gm.	2.3	2.2	2.9	3.0

[7] Monarch, normal plants, 100 per cent; stunted, 98 per cent infections. Markton, normal, 0; stunted, 0 infection. Black Mesdag, normal, 79 per cent; stunted, 68.8 per cent infections. There were approximately 100 plants in each series of normal and stunted plants.

This is to be expected if the fungus does not reach the growing point; it is in the panicle that it produces its characteristic spore masses. On the other hand, if the mycelium remains in the crown node, it may infect the growing points of the tillers.

Reed and Stanton (26), in a study of inheritance of resistance to the smut disease of Markton hybrids, found that in Markton and its hybrids with other varieties a few plants were observed which were described as "blasted." Such plants usually produced infertile spikelets. The spikelets of some plants were rudimentary, those of others were better developed but contained some smut spores. Through the kindness of Dr. Reed seeds of the various F_3 hybrids in which normal infection and blasting occurred were supplied to the writer. They were inoculated, planted, and grown in the greenhouse for three weeks in the fall of 1939. The growing points were then removed and examined histologically. Mycelium was detected in percentages which agreed closely with those given by Reed and Stanton for blasting in these Markton hybrids. It seems, therefore, that blasting may be due to smut infection. Perhaps the smut fungus reached the growing point in these hybrids, disorganized it, but could not complete its life cycle because of internal conditions unfavorable for its sustained growth. There is little cause to doubt that internal factors in Markton are unfavorable for the growth of the mycelium.

DISCUSSION

Much of the discussion of the data bearing on specific points has been presented under the various subdivisions of this paper. It is the purpose here to consider the data as it touches the general problem of systemic infection, with special reference to infection of the oat plant.

From reports in the literature and from this paper, it is apparent that all grades of penetration and infection exist. It would be of benefit to offer a definition of infection for purposes of clarification and discussion; such a definition must be arbitrary.

It is proposed to consider systemic infection in the smuts under three heads, seedling invasion, non-sporulating infection, and sporulating infection. By seedling invasion is meant the infection produced by the penetration of the fungus into the coleoptile or mesocotyl. This invasion of the host does not seem to affect the mature plant adversely under the conditions of germination described here. Under non-sporulating infection one may consider the "latent infection" of Zade (35), and blasting as shown by Reed and Stanton (26). It seems that here the fungus has penetrated much further than in the seedling invasion so that the host is adversely affected. However, little or no sporulation accompanies this condition. In sporulating infection, sporulation of the parasite occurs in the ovaries and their place is taken by the fungus. It is to be noted that there is no sharp line of demarcation here.

This is especially true of sportulating infection; spores may be found in parts of a spikelet, in the entire spikelet or in varying portions of the panicle.

It will be noted that this classification depends on the visible effect of the parasite on the host. It must not be assumed that causation of these different grades of systemic infection by the smut fungus is an indication of the resistance or susceptibility of the host. The environmental factors such as those demonstrated by E. S. Jones (12) and L. R. Jones (13), Reed and Faris (25), and Brandwein (4) must be carefully considered, since under environmental conditions unfavorable for infection even highly susceptible plants may not show evidence of sporulating infection. It is possible that even under favorable environmental conditions a sporulating infection will not take place, since such infection is shown only by those plants inoculated during a limited period. This period occurs early in the life of the oat seedling. Furthermore, conditions favorable for the attainment of seedling invasion must be established during this period. Yet, in highly susceptible varieties, the growing point is susceptible to a sporulating infection even in relatively mature plants. The growing points of mature plants of the resistant Markton are to all practical purposes never susceptible to a sporulating infection. This means that in Markton internal conditions in the growing point are generally unfavorable to the fungus, which cannot establish itself even if it does reach that region. All attempts by this writer to favor the fungus and inhibit the growth of the Markton host and all attempts by Reed (24) with various hosts (by different methods) failed to result in increased infection. The work of Smith and Bressman (29) is to the contrary, however.

It is apparent, therefore, that an internal factor which may be called genetic controls the stages of infection. That this is a specific property is implied in the term "physiologic race" and is supported by the work of Western (32), who shows that different races of smut penetrate to a different extent in Markton, but do not produce sporulating infection. The numerous papers of Reed (20, 21, 22, 23) and the work of Nicolaisen (18) and Reed and Stanton (26) have shown that resistance and susceptibility to various physiologic races of smut are inherited according to Mendelian laws.

This genetic factor is not to be confused with the rate of growth and maturation of the seedling. Since Monarch, Black Mesdag, and Markton exhibit remarkably similar growth rates but differ greatly in their susceptibility, it is evident that growth rates alone may not account for the ability of the plant to escape non-sporulating or sporulating infection. Von Rosenstiel (28) considers this from another viewpoint, for he finds "keine Beziehung zwischen der Lebenskraft der Haferpflanzen und dem Befall durch *U. avenae* besteht." Rapid growth may confer an apparent immunity upon any given susceptible seedling if the fungus, for one reason or another, does not enter or establish itself during the effective period of maximum infec-

tion. This helps to explain unequal infection in susceptible varieties. Markton, however, escapes infection not because of its rapid growth rate, since this does not differ from that of susceptible seedlings, but because of the operation of some internal factor.

It is apparent from the work on genetics, histology, and ecology of infection, considered with the data presented here on effective periods of infection, that there are three main factors which operate in the infection of oat varieties by a given smut fungus. These are: (1) Environmental conditions. (2) The rate of growth and maturation of the seedlings. (3) The genetic factor.

If the internal factor (genetic) and the external factor (environment) are optimum for the growth of the fungus there is a period in which the fungus must enter and develop to produce a maximum sporulating infection.

SUMMARY

The infection of various oat varieties of *Avena sativa* L. by the covered smut, *Ustilago levis* (Kell. and Sw.) Magn., has been studied under controlled environmental conditions.

1. In Monarch, a highly susceptible oat, and in Black Mesdag, a moderately susceptible variety, an effective period of infection has been found in the seedling. In Black Mesdag, this period is shorter than it is in Monarch. Similarly, the period of maximum infection appears to be longer in Monarch than in Black Mesdag.

2. The growth rates of the seedlings of Monarch, Black Mesdag, and Markton during this effective period of infection are remarkably similar. Under environmental conditions suitable for infection, the growth rate between 48 and 96 hours after planting increases approximately 480 per cent over the growth rate during the first 48 hours. In this 48–96-hour period rapidly decreasing infection percentages are found.

3. Unequal infection in susceptible seedlings may be explained on the basis of rate of growth and maturation.

4. The resistance of the Markton oat to infection cannot be explained on the basis of the growth rate. It is probably due to a specific internal factor unfavorable to the development of the smut fungus.

5. It is confirmed that inoculation with the smut fungus under the controlled environment studied here does not result in an adverse effect upon the resistant host.

6. Regional infection studies on the oat seedling indicate that while the oat smut may penetrate and develop in the mesocotyl, this invasion does not result in sporulation. The coleoptile appears to be the structure penetration of which results in highest sporulating infection.

7. It is suggested that three sets of factors control the relationship of oat

and smut fungus. They are: (1) a specific internal factor which may be analyzed on a genetic basis, (2) environmental factors, and (3) a growth factor. While the internal factor controls true resistance, the growth and environmental factors control the amount and extent of infection in susceptible seedlings.

8. The following terminology is suggested which may clarify discussion of systemic infection by oat smut:

(a) Seedling invasion. This occurs in both susceptible and resistant seedlings and need never result in sporulation.

(b) Non-sporulating infection. This appears as "blasting" or "latent" infection but does not result in sporulation.

(c) Sporulating infection. This is evidenced by a sporulation of the fungus in the fruiting structures of the host.

Many thanks are due Dr. George M. Reed, to whom the writer is indebted for materials, field space, and other aid without which this work could not have been accomplished.

BROOKLYN BOTANIC GARDEN,
AND
WASHINGTON SQUARE COLLEGE,
NEW YORK UNIVERSITY.

Literature Cited

1. **Arland, A.** 1924. Der Haferflugbrand *Ustilago Avenae* (Pers.) Jens. Biologische Untersuchungen mit besonderer Berücksichtigung der Infections- und Anfälligkeitsfrage. Bot. Arch. **7**: 70–111.
2. **Beyer, A.** 1927. Zur Keimungsphysiologie von *Avena Sativa*. Ber. Deuts. Bot. Ges. **45**: 179–187.
3. **Brandwein, P. F.** 1937. Experiments on latent infection of resistant varieties by the loose and covered smut of oats. Bull. Torrey Club **64**: 433–444.
4. ————————. 1938. The emergence of smut-inoculated oat seedlings through sand and loam soil. Bull. Torrey Club **65**: 477–483.
5. **Brefeld, O.** 1888. Recent investigations of smut fungi and smut diseases. Nachricht. Klub der Landwirthe zu Berlin, Nos. 220–222. (Trans. by E. F. Smith, Jour. Mycol. **6**: 1–8, 59–71, 153–164. 1890.)
6. **Brefeld, O. & Falck, R.** 1905. Die Blüteninfektion bei den Brandpilzen und die natürliche Verbreitung der Brandkrankheiten. *In* Brefeld, O. Unters. Gesamt. Myk. **13**: 1–74.
7. **Diehl, O.** 1925. Experimentelle Untersuchungen über die Lebensweise und Bekämpfung des Haferflugbrandes. Bot. Arch. **11**: 146–199.
8. **Faris, J. A. & Reed, G. M.** 1925. Modes of infection of Sorghums by loose kernel smut. Mycologia **17**: 51–67.
9. **Gage, G. R.** 1927. Studies of the life history of *Ustilago Avenae* (Pers.) Jens. and of *Ustilago levis* (Kell. and Swing.) Magn. Cornell Exp. St. Mem. 109: 3–35.
10. **Hiltner, L.** 1908. Über die Abhängigheit der Brandanfälligkeit des Getreides von dessen Keimungsenergie und Entwicklungsgeschwindigkeit. Prakt. Blatt. Bayer. Landesanst. **6**: 67–69.

11. **Hubbard, V. C. & Stanton, T. R.** 1934. Influence of smut infection on plant vigor and other characters in smut-resistant oat varieties. Jour. Agr. Res. **49:** 903–908.
12. **Jones, E. S.** 1923. Influence of temperature, moisture and oxygen on spore germination of *Ustilago avenae*. Jour. Agr. Res. **49:** 903–908.
13. **Jones, L. R.** 1896. Some observations regarding oat smut. Vt. Exp. Sta. Ann. Rept. **9:** 106–112.
14. **Kolk, M.** 1930. Relation of host and pathogen in the oat smut, *Ustilago Avenae*. Bull. Torrey Club **57:** 443–507.
15. **Kühn, J.** 1874. Die Art des Eindringens der Keimfaden in die Nahrpflanze. Ber. Sitz. Nat. Ges. Halle **1874:** 5–7.
16. **Lang, W.** 1912. Zum Parasitismus der Brandpilze. Jahresb. Verein, Angew. Bot. **10:** 172–180.
17. **McAlpine, D.** 1910. The smuts of Australia. Dept. Agr. Victoria. i–vi, 1–288.
18. **Nicolaisen, W.** 1931. Beitrag zur Immunitätszuchtung des Hafers gegen *Ustilago avenae* (Pers.) Jens. Zeitschr. Zücht. A **16:** 255–278.
19. **Reed, G. M.** 1924. Physiologic races of oat smuts. Am. Jour. Bot. **11:** 483–492.
20. ————————. 1928. The inheritance of resistance of oat hybrids to loose and covered smut. Ann. N. Y. Acad. Sci. **30:** 129–176.
21. ————————. 1932. Inheritance of resistance to loose and covered smut in hybrids of Hull-less with Early Gothland and Monarch oats. Am. Jour. Bot. **19:** 273–301.
22. ————————. 1934. Inheritance of resistance to loose and covered smut in hybrids of Black Mesdag with Hull-less, Silvermine, and Early Champion oats. Am. Jour. Bot. **21:** 278–291.
23. ————————. 1935. Inheritance of resistance to loose smut in hybrids of Fulghum and Black Mesdag oats. Bull. Torrey Club **62:** 177–186 illus.
24. ————————. 1938. Influence of the growth of the host on smut development. Proc. Am. Phil. Soc. **79:** 303–326.
25. **Reed, G. M. & Faris, J. A.** 1924. Influence of environal factors on the infection of sorghums and oats by smuts. II. Experiments with covered smut of oats and general considerations. Am. Jour. Bot. **11:** 579–599.
26. **Reed, G. M. & Stanton, T. R.** 1938. Inheritance of resistance to loose and covered smuts in Markton oat hybrids. Jour. Agr. Res. **56:** 159–175.
27. **Roesch, A.** 1926. Studien über den Haferflugbrand, *Ustilago Avenae* (Pers.) Jens. und den Glatthaferbrand, *Ustilago Perennans*, Rostr. mit besonderer Berücksichtigung der Immunitätsfrage beim Haferflugbrand. Bot. Arch. **13:** 382–432.
28. **Rosenstiel, K. von.** 1929. Untersuchungen über die Widerstandsfähigkeit von Haferarten und sorten gegen Haferflugbrand *Ustilago avenae* (Pers.) Jens. und ihre Vererbung. Phytopath. Zeitschr. **1:** 317–360.
29. **Smith, D. C. & Bressman, E. N.** 1931. Susceptibility of Markton and other varieties of oats to covered smut (*Ustilago levis*). Jour. Am. Soc. Agron. **23:** 465–468.
30. **Stevens, H.** 1936. The effect of latent infection on the smut-resistant Markton oat. Jour. Am. Soc. Agron. **28:** 711–713.
31. **Tapke, V. F.** 1939. Influence of environment, after seedling emergence, on loose smut of oats and covered smut of barley. Phytopathology **29:** 22–23. (Abstract)
32. **Western, J. H.** 1936. The biology of oat smuts. IV. The invasion of some susceptible and resistant oat varieties, including Markton, by selected biological species of smut, *Ustilago avenae* (Pers.) Jens. and *Ustilago kolleri* Wille. Ann. App. Biol. **23:** 245–263.

33. **Wolff, R.** 1873. Beitrag zur Kenntniss der Ustilagineen. Bot. Zeit. **31**: 657–661.
34. **Zade, A.** 1922. Experimentelle Untersuchungen über die Infection des Hafers durch Haferflugbrand *Ustilago Avenae* (Pers.) Jens. Fühlings Landw. Zeit. **71**: 393–406.
35. ————————. 1932. Neue Untersuchungen über den latenten Pilzbefall und seinen Einfluss auf die Kulturpflanzen. Fortschr. Landw. **7**: 529–532.

TAR SPOT OF AMERICAN HOLLY

E. S. LUTTRELL[1]

(WITH SIXTEEN FIGURES)

INTRODUCTION

In 1938 Wolf and his associates (14) reported the occurrence in the Duke Forest of a tar spot disease on the foliage of American holly, *Ilex opaca* Ait., caused by a fungus commonly called *Macroderma Curtisii* (B. & Rav.) v. Höhn., and suggested that this disease deserved special study since it has not been dealt with previously. Such a study was undertaken and the results are recorded herein. Besides providing a description of the disease, the purpose of this paper is to present an account of the structure and development of the pathogen which will aid in determining its systematic position and at the same time extend the present knowledge of developmental morphology in the Phacidiaceae.

APPEARANCE OF THE DISEASE

The appearance of tiny yellow spots on holly leaves during late May constitutes the first symptom of the disease. At this stage the spots cannot readily be distinguished from those produced by certain sucking insects unless freehand sections of lesions are examined for the mycelium of the fungus. During early summer the yellow spots slowly increase in area. By the middle of July the central portion of each spot becomes reddish brown and the discolored area gradually enlarges until only a narrow border of yellow tissue remains. By fall the discoloration has deepened to shining black, and flat cushion-shaped stromata have developed beneath the epidermis. Frequently pairs of stromata develop in a single lesion, one near the upper surface and the other near the lower surface, and sometimes elongated boat-shaped ones form independently on the ventral surface of the midrib. By November the stromata on the upper leaf surface have attained diameters of 1–4 mm., while those on the lower surface are somewhat smaller. Differentiation of the stromata proceeds slowly throughout the winter months, and by April the orange-red apothecial discs are matured and are exposed by the rupture of the outer layers. After dehiscence the surrounding leaf tissues die as a result of desiccation, forming a buff col-

[1] I am grateful to Dr. Frederick A. Wolf, under whose direction this study was conducted, for suggesting the problem and for his criticism and assistance in the preparation of the manuscript. I am also indebted to Dr. David H. Linder, Harvard University, for information about collections in the Farlow Herbarium; to Dr. Sophie Satina, Department of Genetics, Carnegie Institution of Washington, for furnishing an abstract of her paper on *Phacidium repandum*; and to Dr. Lewis E. Anderson, Duke University, for taking the photomicrographs which appear in figs. 2–7.

ored necrotic area which bears the exhausted stroma. These necrotic areas may finally drop out, leaving perforations. Infected leaves usually remain on the tree for the normal period of two years, the unaffected portions continuing to function, but occasionally severely infected leaves may become entirely yellow and moribund by the end of the first year or parts or all of the leaf may have died before that time. The fungus does not continue development after the infected tissue of such leaves becomes necrotic.

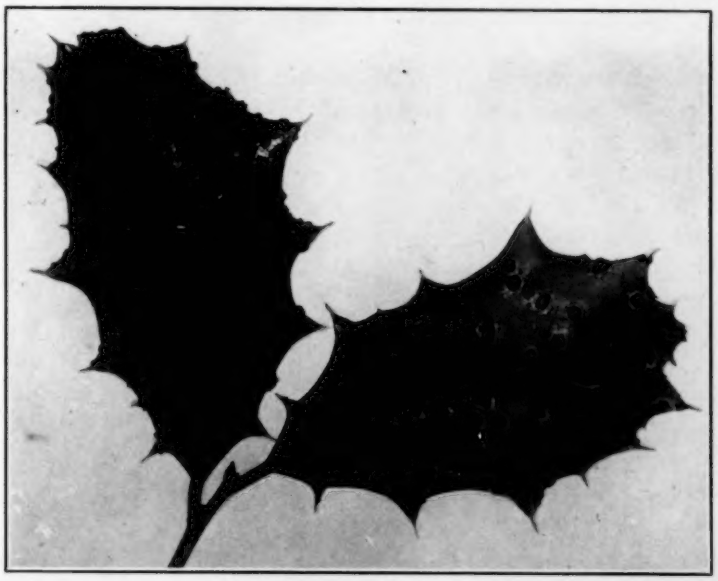


FIG. 1. Leaves of American holly infected with *Phacidium Curtisii*. The black stromata have been ruptured so as to expose the orange-red apothecial discs of the fungus.

RANGE AND IMPORTANCE OF THE DISEASE

The fungus known as *Macroderma Curtisii* is limited to a single host species, *Ilex opaca*. It has been collected in many parts of North Carolina from the sea coast to the mountains, under a wide range of environmental conditions. Collections have also been made in Virginia, Maryland, and South Carolina. There are specimens in the Farlow Herbarium from Massachusetts, Maryland, West Virginia, North Carolina, Georgia, and Florida. It seems likely, therefore, that the range of this fungus coincides with that of the American holly.

Trees of all sizes are attacked by the tar spot fungus, the age of the tree making little difference in severity of attack or freedom from disease. As a rule there is no premature defoliation. Nevertheless, infection results in a decrease in photosynthetic area of the leaves. This impairment in photosyn-

thetic tissue has not been observed to be of consequence on mature trees although it may cause a significant decrease in vigor of severely infected seedlings.

STRUCTURE AND DEVELOPMENT OF THE PATHOGEN

Development of the fungus was followed in free-hand sections cut from infected tissues supported in pith. The sections were mounted in water or in lacto-phenol in which 0.6 per cent cotton blue had been dissolved. Such preparations proved to be superior to paraffin sections for studying the morphology and host relations of the fungus, but microtome sections were necessary for determining cytological details. At appropriate intervals throughout the year fixations of infected tissues were made with Navashin's fluid, alcohol-acetic solution, or a fixative made of 4 parts of a saturated solution of picric acid in dioxan, 1 part of acetic acid, and 4 parts of absolute alcohol. The last solution gave satisfactory results and proved very convenient, since the material could be quickly passed from the fixative through several changes of pure dioxan into paraffin. Storage in dioxan did not seem to be harmful to the tissues. After the material was embedded in paraffin, sections were cut 5 μ thick. The sections were stained either with a combination of safranin followed by fast green in clove oil or with Haidenhain's iron alum haematoxylin counterstained with aqueous safranin or orange G in clove oil.

The stroma. Cross sections of the lesions found in early summer show that the host cells from the upper to the lower epidermis are occupied by mycelium of the fungus, which is entirely intracellular. The hyphae, which are about 3 μ in diameter and consist of thin-walled cylindrical cells with prominent nuclei, branch and coil within the host cells and readily penetrate the cell walls. Even vascular cells are occasionally penetrated. The infected cells, however, are not killed. The presence of the reddish discoloration in lesions during July is indicative of the initiation of stromata. Formation of the stroma is first evident within the cells of the upper epidermis. The lateral

Explanation of Figures 2–7

FIG. 2. A portion of the hymenium of *Phacidium Curtisii* with asci in various stages of development.

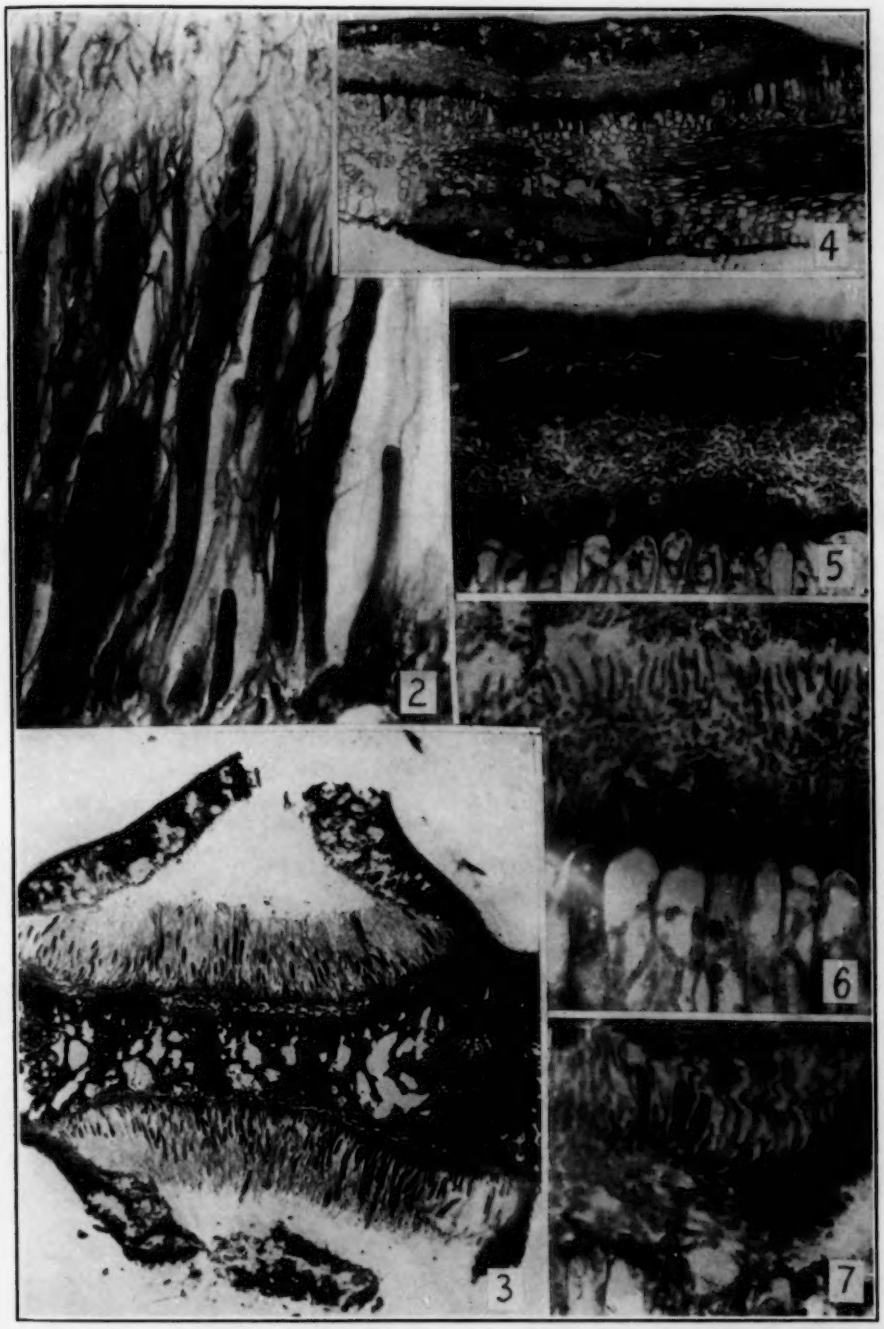
FIG. 3. A section of a holly leaf bearing fertile stromata on both upper and lower surfaces. Each stroma contains a single apothecium which is exposed when the overlying stroma ruptures.

FIG. 4. A section of a leaf showing stromata within which apothecia have just begun to form.

FIG. 5. An enlarged view of a stroma before the formation of the apothecium has begun. Note that it is differentiated into dark-colored, compact basal and outer portions and a median loose plectenchymatous layer within which the apothecium will develop.

FIG. 6. A section of the median portion of a stroma in which a layer of erect hyphae, the paraphyses, is developing.

FIG. 7. Young asci growing up among the paraphyses.


4
5
2
6
3
7

walls of these cells are ruptured and dissolved, and the mycelium forms a continuous plectenchymatous stroma in the space thus formed between the outer epidermal walls and the palisade layer. Within the central part of the infected area the palisade cells are gradually disintegrated until the stroma extends down to the spongy mesophyll. The stromata contain large druses of calcium oxalate which were formed within hypertrophic epidermal and mesophyll cells (fig. 8). A few such crystal-bearing cells are always found scattered throughout healthy leaves, but as a result of infection an inordinate number of these cells is produced. During fall the stromata become differentiated into three layers (figs. 4, 5): (1) An outer layer in which the hyphae are thick-walled and very compactly arranged; this tissue and the adjacent epidermal walls become impregnated with a dark purplish pigment which is responsible for the deep black color of stromata when observed macroscopically, and the cuticle over these areas takes on a yellowish hue. (2) A median layer composed of a loose plectenchyma of hyaline hyphae. (3) A basal layer made up of partially disintegrated mesophyll cells filled with thick, dark-colored hyphae.

The hypophyllous stromata are formed in the same manner within the lower epidermis and adjacent spongy mesophyll. Less disintegration of leaf tissue accompanies their formation, however, because they are smaller and because of the large proportion of intercellular spaces in the spongy mesophyll. Moreover, the dark basal layer of the stroma is mostly lacking. The presence of stromata is accompanied by an increase in thickness of the infected areas, which usually become almost twice as thick as normal portions of the same leaf. Just prior to formation of the apothecia the hypophyllous stromata may be about 200 μ in thickness, while those on the upper surface of the leaf are approximately 300 μ thick.

The spermogonia and archicarps. Spermogonia mature during the latter part of August but do not occur in all stromata. They are visible macroscopically as brilliant red spots, one centrally situated on each stroma. In

Explanation of Figures 8–16

FIG. 8. Diagrammatic drawing showing the positions of the spermogonium and the archicarps in the stroma of *Phacidium Curtisii*. The stroma has not yet differentiated into the three layers found in later stages. The large druses included in the stroma were formed within hypertrophic host cells.

FIG. 9. A section of the spermogonium showing the formation of spermatia.

FIG. 10. Archicarps of *Phacidium Curtisii*.

FIG. 11. The origin of the asci from binucleate cells located in the stroma beneath the hymenium.

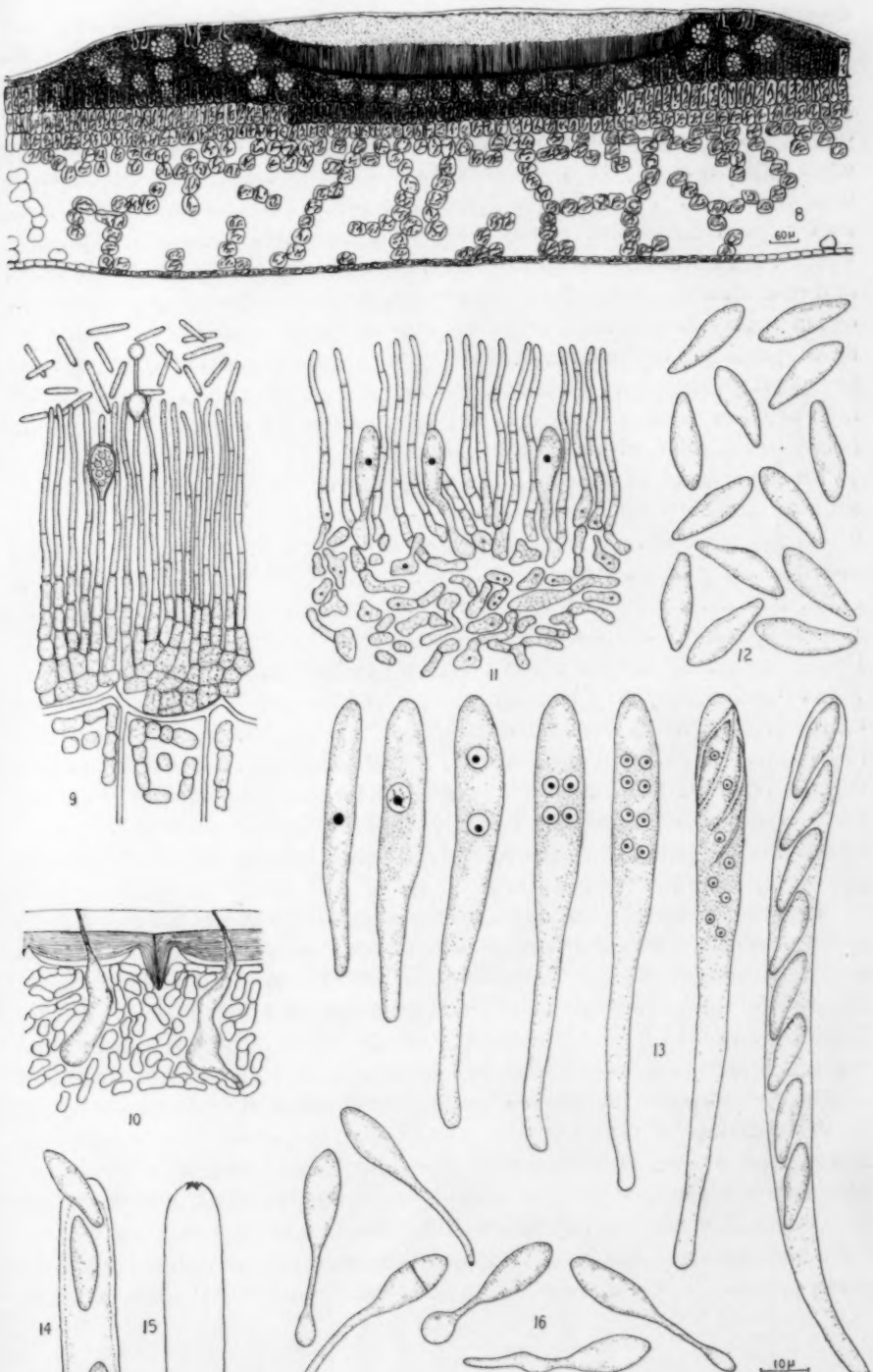
FIG. 12. Mature ascospores.

FIG. 13. Stages in the formation of ascospores.

FIG. 14. Ascospore discharge.

FIG. 15. An empty ascus showing at the apex the pore through which the ascospores emerged.

FIG. 16. Germinating ascospores.


8
60μ
9
10
11
12
13
14
15
16
10μ

cross section the spermogonium appears as a flat, saucer-shaped structure in the outer layer of the stroma, covered only by the outer epidermal walls and leaf cuticle (fig. 8). It is therefore acervular in structure. The basal portion of the spermogonium is composed of a pseudoparenchymatous layer of polygonal cells from the surface of which arises a palisade of slender erect hyphae, the spermatiophores. They are about 40 μ long and 1.3 μ in diameter (fig. 9). Bacilliform spermatia, 7–12 × 1.3 μ, are abstricted from their tips. Interspersed among the spermatiophores are erect hyphae in which are intercalated large spherical cells (fig. 9) which are thick-walled and contain numerous refractive globules. A single hypha may contain only one of these enlarged cells, or several may be disposed at intervals along the apical portion. The function of these hyphae is uncertain, but they probably represent paraphysoid elements. When the spermogonium is mature the area between the tips of the spermatiophores and the outer epidermal walls of the leaf is filled with a mass of spermatia.

In the same stroma, beyond the periphery of the spermogonium, deeply staining filaments that project through the cuticle may be found (fig. 8). These filaments have been traced downward through the epidermal walls and into the plectenchyma of the stroma, where each swells into an elongated bulbous structure which is non-septate and contains several nuclei (fig. 10). This structure is interpreted as an archicarp, composed of an enlarged ascogonial portion which tapers above into an elongated trichogyne. It is approximately 40 μ long and ranges in diameter from 0.3 μ at the tip of the trichogyne to 7 μ in the basal part of the ascogonium. Numerous archicarps are found in each stroma. Sometimes hyphal branches are seen to arise from the base of the ascogone and penetrate the stroma, but it has been impossible to trace them farther. Apparently archicarps develop only in conjunction with spermogonia, for in those stromata which lack spermogonia they have not been observed.

When spermogonia and archicarps first appear, the stroma shows little differentiation. Gradually the upper portions containing the archicarps become more compact and darker colored, and the archicarps disintegrate. Meanwhile the exhausted spermogonium becomes filled with a mass of plectenchyma which cements the epidermal cell walls to the stroma, and the whole structure becomes heavily impregnated with purplish pigment.

The apothecium. The apothecia are formed within the plectenchymatous median portion of the stromata. Sections of the stroma made in early March show a layer of erect hyphae, the paraphyses, developing within the plectenchyma (figs. 4, 6). The paraphyses, which arise as branches from the hyphae of the stroma and are from the first free at the tips, press against the overlying stromatic layer and by their continued growth split it in a plane parallel to the surface. When they have reached a length of about

40 μ, young asci begin to push up among them from the hypothecial layer (fig. 7). The asci originate directly from binucleate cells in the hypothecium. Each binucleate cell produces a protuberance on the side toward the hymenium. Meanwhile the two nuclei fuse within the cell and the fusion nucleus migrates into the protuberance, which elongates to form the ascus (fig. 11). In early stages a large nucleolus is the conspicuous part of the nucleus (fig. 13), while later the rest of the nucleus becomes more evident, appearing as a hyaline area around the nucleolus.

The ascus remains uninucleate until it reaches a length of 60–80 μ. The first division of the nucleus is oriented parallel to the longitudinal axis of ascus, so that the two daughter nuclei are located one above the other. These the divide in a plane perpendicular to the plane of the first division to form four nuclei arranged in pairs, and the four nuclei divide to form eight, likewise arranged in pairs one above the other in the ascus. Cleavage furrows now appear in the cytoplasm surrounding the nuclei and cut out the ascospores. Cleavage may be initiated first at the apex of the ascus and progress towards the base, or it may delimit first the spores at the basal end. The spores increase in size at the expense of the epiplasm until, when mature, they measure 19–24 × 5–6 μ and are hyaline, one-celled, and narrowly obovate in shape (fig. 12). When first delimited they are more or less biseriately arranged, but they are always imbricately uniseriate by the time the ascus is mature (fig. 13).

The ascus is cylindrical, 100–160 × 9 μ. The upper part, containing the spores, is slightly enlarged, while the basal portion tapers into an elongated stalk in which the cytoplasm is sparse and vacuolate (fig. 2). At maturity the ascus ruptures at the apex, and the ascospores are expelled through the irregular pore (figs. 14, 15). Asci in all stages of development may be found in each apothecium (fig. 2). The asci, paraphyses, and cells of the hypothecium become filled with golden-orange oil globules which are responsible for the brilliant orange-red color of the disc as seen macroscopically.

At about the time that the first ascospores are mature the overlying portion of the stroma is ruptured, and the hymenium is exposed (figs. 1, 3). Dehiscence consists of a mechanical splitting resulting from the pressure exerted by the expanding hymenium. The first evidences of dehiscence are visible macroscopically about the middle of March, when an irregular crack may appear across the surface of the stroma. From this crack fissures radiate towards the margin of the stroma and deepen until the outer layer of the stroma is completely severed. The ruptured stromatic tissue is turned back by the expanding asci and remains as a fringe of irregular teeth bordering the apothecium. These teeth are hygroscopic, closing over the hymenium when dry and opening when moist to expose it again.

Ascospore germination. Ascospores for use in studies on germination were obtained by arranging leaves bearing fully developed apothecia so that the ascospores were discharged upon an agar plate or into a drop of distilled water on a glass slide. The ascospores germinate within 12 hours by the production of a single germ tube about 1.5 μ in diameter, usually from the more acute end of each spore (fig. 16). Occasionally the ascospore may be divided into two cells by the formation of a transverse wall. In culture the hyphae do not become septate, and after reaching a length of about 25 μ they cease to grow, their tips usually swelling to form spherical vesicles. Further development in culture has not been obtained.

TAXONOMY OF THE PATHOGEN

The pathogen was originally described by Berkeley and Ravenel (North Am. Fungi no. 780) as *Rhytisma Curtisii*. Their description (8) was, however, inadequate. In 1916 Theissen (10) supplied a more complete description of the fungus, and stated, moreover, that it should not be retained in the genus *Rhytisma* because of several important differences. He noted that in other species of *Rhytisma* the ascospores are long, thread-like, and septate, and the apothecia are grouped within the stromata, while in *Rhytisma Curtisii* the spores are unicellular and elliptical and each stroma contains only a single apothecium. Although he did not make any change in name, Theissen suggested, on the basis of the characters just enumerated, that this fungus might better be placed in the genus *Phacidium* but might be found to belong to *Trochilla*.

In 1917 von Höhnel (3), without emendation of Berkeley and Ravenel's description, designated *Rhytisma Curtisii* as the type of a new genus, *Macroderma*. At the same time he divided the order Phacidiales into five families based entirely on the position of the ascocarp within the host tissue. *Macroderma* was placed in his family Dermopeltinaceae, which included those Phacidiales which develop within the epidermis. *Phacidium*, on the other hand, was placed in the Phacidiaceae, a family composed of those Phacidiales which develop beneath the epidermis or more deeply within the host tissue.

Von Höhnel's erection of the new genus, *Macroderma*, has been criticised by Nannfeldt (7, pp. 240–241), who was able to find only slight differences between *Macroderma Curtisii* and *Phacidium lacerum* Fr., the species which von Höhnel had selected as the type of *Phacidium*. He found that *M. Curtisii* differs from *P. lacerum* in the following respects: the stromata of *M. Curtisii* are larger and more strongly developed, stromata occur on the lower as well as the upper surface of the leaves, and the tips of the asci do not stain blue with iodine. He provisionally treated *Macroderma* as a separate genus, being extremely doubtful whether there is any sharp boundary between it and *Phacidium*.

Theissen (10) described the stromata of *M. Curtisii* as resting on the palisade layer which, according to him, was stained light brown but was otherwise unaffected. Von Höhnel's (3) inclusion of *Macroderma* in the Dermopeltinaceae was based on this same conception of the relation of the stroma to the host tissue. As has been shown by Nannfeldt (7) and by my own studies, however, formation of the stroma always involves more or less disintegration of the mesophyll tissues, and the stroma usually extends to the spongy mesophyll. If von Höhnel's key is followed the fungus must therefore be placed in the Phacidiaceae, where it falls naturally into the genus *Phacidium*.

The above mentioned differences between *Macroderma* and *Phacidium* do not seem to me to be of sufficient importance to warrant their separation. In other more fundamental characters they agree closely. In both *Phacidium* and *Macroderma* the apothecia are produced singly within separate stromata which are embedded in the host tissue. At maturity they are exposed by the stellate rupture of the overlying stroma and the host tissue with which it is fused. The asci are cylindrical, and the ascospores are elliptical, continuous, and hyaline. The paraphyses are slender and unbranched. *Macroderma Curtisii* is therefore transferred to the genus *Phacidium*, family Phacidiaceae. Since no adequate diagnosis has previously been published, the description is emended as follows:

Phacidium Curtisii (B. & Rav.) Luttrell, comb. nov. amend. *Rhytisma Curtisii* B. & Rav. North Am. Fungi no. 780. *Macroderma Curtisii* (B. & Rav.) v. Höhn. Ber. Deutsch. Bot. Gesel. **35**: 422. 1917.

Stromatibus in maculis innatis, planis vel discoideis, 1–4 mm. diam., atronitidis, intraepidermalibus atque foliorum in cellulis aliis; ascomatibus in verno stromatibus innatis, solitariis, amphigenis, demum irregulariter vel rimose laceratis; disco rubro-aurantiaco, 1–4 mm. diam.; ascis clavato-cylindraceis, ex hypothecio hyalino oriundis, octosporis, 110–160 × 9 μ; paraphysibus filiformibus, simplicibus, 100–160 × 1.3 μ; ascosporis oblique monostichis, fusoideis vel obovatis, continuis, hyalinis, 19–24 × 5–6 μ.

In aestate et in autumno spermogoniis atque carpogoniis efformantibus; spermogoniis erumpentibus, planis, miniatis; spermatiophoris simplicibus, interdum nodosis, 40 × 1.3 μ; spermatiis bacilliformibus, hyalinis, 7–12 × 1.3 μ.

Habitat in foliis vivis *Ilicis opacae*.

DISCUSSION

Apparently the Phacidiaceae are a group whose members exhibit more or less reduction in sexuality from forms which produce functional spermatia and archicarps. Higgins (2), in a study of several species of *Coccomyces*, found within the stromata archicarps, each of which consisted of a coiled ascogonial portion of enlarged uninucleate cells and an elongated trichogyne extending through the acervulus in which microconidia were produced. In a subsequent investigation of one of these species, *C. hiemalis* Hig-

gins, Backus (1) was able to show that the spermatia become attached to the tips of the trichogynes. Although actual passage of the spermatial nucleus down the trichogyne and the production of ascogenous hyphae by the ascogonial cells were not observed, it is highly probable that in *Coccomyces* fusions between spermatia and trichogynes accomplish fertilization and are a necessary preliminary to the development of asci. Archicarps similar to those of *Coccomyces* were described in two species of *Diplocarpon* by Wolf (13). The trichogynes were found to extend between the epidermal cells of the host and to develop at the same time that microconidia appeared in greatest abundance in the acervuli. It seems likely, therefore, that in *Diplocarpon* the processes of fertilization and formation of asci are the same as in *Coccomyces*.

In *Rhytisma Acerinum* (Pers.) Fr. (Jones 4), *Lophodermium hysterioides* (Pers.) Sacc. (Likhité 6), and *Phacidium repandum* Alb. et Schw. (Satina 9), the asci are produced parthenogamously. Fertilization always results from fusions between cells of a multicellular archicarp brought about by dissolution of the transverse walls. Spermatia are produced by *R. Acerinum,* but they are non-functional, the 5–6-celled archicarps being formed later and being deeply buried within the stroma. In *L. hysterioides* the archicarps, which also are completely immersed in the stroma, consist of as many as 40 cells, and no spermatia are produced. In *P. repandum*, on the other hand, the coiled basal portions of the archicarps terminate in elongated trichogynes which extend through stomata of the host, but there are no spermatia and the trichogynes are believed (9) to be functionless.

In *Lophodermium pinastri* (Schrad.) Chev., Jones (5) encountered a more complex situation. Among the spermatiophores, which are formed in acervuloid spermogonia beneath the partially disintegrated epidermis, he found bulbous, non-septate structures tapering at the apices into elongated beaks which he interpreted as receptive female organs. He found that the spermogonia later may be converted into apothecial stromata, and the ascogenous hyphae found in these stromata he considered to be derived from the above-mentioned archicarps after spermatiation. He discovered, however, that other apothecial stromata could arise independently of spermogonia and that in these stromata no archicarps were formed. Jones concluded that the ascogenous hyphae in the latter stromata originated from fusions between vegetative hyphae.

As has been shown in this paper, practically the same phenomena which Jones describes in *L. pinastri* are found in *Phacidium Curtisii*. My interpretation of the facts, however, deviates in some respects from that of Jones, for it seems unlikely that a single fungus would exhibit such diverse sexual phenomena as he indicated for *L. pinastri*. If apothecia may be produced in some stromata without the intervention of spermatia and archicarps, it is difficult to believe that these organs are essential in any stroma. Although

archicarps and spermatia are commonly present in *P. Curtisii*, they have not been demonstrated to be functional, and from the evidence now available it is concluded that the ascogenous cells always arise by some pseudomictic process from undifferentiated hyphae of the stroma.

Early in the development of the apothecium in *Phacidium Curtisii* a layer of paraphyses is formed across the median portion of the stroma. The paraphyses arise as hyphal branches which are from the beginning free at the tips, and the asci later grow up among them from the hypothecium. The hymenium therefore consists of asci and true paraphyses. The development of the apothecium is exactly like that in *Lophodermium Berberidis* (Schleich.) Rehm as described by Nannfeldt (7, pp. 234–236). Jones' (4) account of apothecial formation in *Rhytisma Acerinum* is, however, slightly different. According to this author the archicarps of *R. Acerinum* are formed within a layer of vertical hyphae in the interior of the stroma. Later these hyphae break away from the overlying stroma and cannot be distinguished from the true paraphyses which grow up among them from the stroma beneath. Nannfeldt (7, p. 217) has questioned the occurrence of true paraphyses and interthecial threads in the same hymenium, and he is supported by the present observation on *Phacidium Curtisii*.

The rupture of the outer part of the stroma in the Phacidiaceae may be caused by mechanical pressure of the expanding hymenium within, or this process may be assisted by specialized opening mechanisms in the roof of the stroma. In *Phacidium Curtisii* as in *P. repandum* (Satina 9), *Diplocarpon* spp. (Wolf 11, 12), and *Coccomyces* spp. (Higgins 2), no specialized means of opening is found. In *Rhytisma Acerinum* and in *Lophodermium pinastri*, on the other hand, Jones (4, 5) reports the presence of structures which function in the splitting of the stroma, and Nannfeldt (7, pp. 219, 235, 238) described similar mechanisms in several other species of *Lophodermium*.

SUMMARY

A tar spot disease is common on the leaves of *Ilex opaca* Ait. in North Carolina, and apparently is found throughout the range of the tree. The disease produces yellow lesions which become necrotic by the end of the first year, but the unaffected parts of the leaves usually continue to be functional for the normal period of two years. Infection results in a decrease in photosynthetic area which may affect the vigor of seedlings.

On the basis of its structure and development the pathogen, first called *Rhytisma Curtisii* B. & Rav. and later *Macroderma Curtisii* (B. & Rav.) v. Höhn., is transferred to the genus *Phacidium*, family Phacidiaceae, and the description is emended.

The mycelium of *P. Curtisii* is at first entirely intracellular. Later it forms a plectenchymatous stroma in the space resulting from a partial dissolution of the epidermal and adjacent mesophyll tissues.

The black, disc-shaped stromata are formed singly on the upper surface of each lesion, or in pairs, one on the upper and one on the lower leaf surface. During fall archicarps and acervuloid spermogonia are formed within some of the stromata. They have not, however, been demonstrated to be functional. In spring a layer of paraphyses develops across the median portion of the stroma, and the asci subsequently grow up among the paraphyses from the hypothecium. They arise directly from binucleate ascogenous cells which probably have a pseudomictic origin.

The covering layer of the stroma and the outer epidermal cell walls with which it is fused are split in a stellate manner as a result of the pressure exerted by the expanding hymenium, and the ruptured tissues remain as a peripheral fringe of hygroscopic teeth around the orange-red apothecial disc. The ascospores, which are discharged through an irregular pore in the apex of the ascus, germinate readily, forming short germ tubes, but further growth in culture was not obtained.

Development of *P. Curtisii* accords in essential features with that of other members of the family Phacidiaceae.

DEPARTMENT OF BOTANY, DUKE UNIVERSITY
DURHAM, NORTH CAROLINA

Literature Cited

1. **Backus, M. P.** 1934. Initiation of the ascocarp and associated phenomena in *Coccomyces hiemalis*. Contr. Boyce Thompson Inst. **6**: 339–379.
2. **Higgins, B. B.** 1914. Contributions to the life history and physiology of *Cylindrosporium* on stone fruits. Am. Jour. Bot. **1**: 145–173.
3. **Höhnel, F. von.** 1917. System der Phacidiales v. H. Ber. Deuts. Bot. Ges. **35**: 416–422.
4. **Jones, S. G.** 1925. Life history and cytology of *Rhytisma acerinum* (Pers.) Fries. Ann. Bot. **39**: 41–76.
5. ————————. 1935. The structure of *Lophodermium pinastri* (Schrad.) Chev. Ann. Bot. **49**: 699–728.
6. **Likhité, V.** 1926. Recherches sur le développement et la biologie de quelques Ascomycètes. Rev. Gén. Bot. **38**: 5–30, 95–106.
7. **Nannfeldt, J. A.** 1932. Studien über die Morphologie und Systematik der nichtlichenisierten inoperculaten Discomyceten. Nova Acta Soc. Sci. Upsal. IV **8** (2): 1–368.
8. **Saccardo, P. A.** 1889. Sylloge Fungorum **8**: 756.
9. **Satina, S.** 1921. Studien über die Entwicklung der Haupt- und Nebenfruchtformen bei *Phacidium repandum* Alb. et Schw. Zeitschr. Russ. Bot. Ges. **4**: 95–104.
10. **Theissen, F.** 1916. Verschiedene Mitteilungen. Ann. Myc. **14**: 263–273.
11. **Wolf, F. A.** 1912. The perfect stage of *Actinonema rosae*. Bot. Gaz. **54**: 218–234.
12. ————————. 1924. Strawberry leaf scorch. Jour. Elisha Mitchell Soc. **39**: 141–163.
13. ————————. 1926. The leaf scorch disease of strawberries. N. C. Exp. Sta. Tech. Bull. 28: 1–16.
14. **Wolf, F. A., Garren, K. H. & Miller, J. K.** 1938. Fungi of the Duke Forest. Duke Univ. School of Forestry Bull. 2: 1–122.

TEGILLUM, A NEW GENUS OF THE UREDINALES[1]

E. B. MAINS

(WITH SEVEN FIGURES)

In 1936, the writer collected a rust on a species of *Vitex* in the El Cayo district of British Honduras. This has pycnia, uredinia, and telia. The telia contain one-celled, hyaline, sessile teliospores borne in groups on laterally free sporogenous cells. The urediniospores are angular with the pores in the angles. Both the uredinia and telia are bordered by dark brown paraphyses. The genus *Olivea* is immediately suggested and specifically *O. scitula* described by Sydow (1937) on *Vitex Cienkowskii* from Sierra Leone. However further study revealed several important points of difference.

The genus *Olivea* was proposed by Arthur (1917) with *O. capituliformis* (Henn.) Arth. as the type species on *Alchornea* sp. He also described *O. Petitiae* on *Petitia domingensis*. Sydow (1937) has added a third species, *O. scitula* on *Vitex Cienkowskii*.

In his description of the genus Arthur (1917, 1925) states that the aecia are subepidermal and that the other sori are subcuticular. The aecia are described as deep seated in the host tissue and without peridia; the uredinia as minute, from stem-like bases expanding into globose masses of paraphyses. It is stated that the teliospores are one-celled, cylindric, and colorless.

Dietel (1924) differs in his interpretation of the genus in that the uredinia and telia are considered to be subepidermal and the teliospores arise in groups from fertile hyphae.

Through the kindness of G. B. Cummins and H. Sydow, I have been able to study collections of the three species. The following conclusions have been reached. The pycnia, which are known only for *O. capituliformis*, are subcuticular. The aecia, which are known only for *O. capituliformis*, are subepidermal. Their deep-seated position is due to the pronounced enlargement of the epidermal cells which overlie and surround them. The uredinia and telia, which occur in all three species, are superficial. It is difficult to determine the exact manner of their development. They appear to originate from compact masses of mycelia in the substomatal intercellular spaces. The hymenial cells probably emerge through the stomata, obliterating them. The uredinia and telia therefore develop mostly above the epidermis, the encircling peripheral paraphyses from the narrow bases producing the characteristic superficial, basket-like sori.

The rust from British Honduras shows several important points of difference. All the sori from the gametophytic mycelium (pycnia, uredinia,

[1] Paper from the Department of Botany and the Herbarium of the University of Michigan. This investigation has been supported with funds from the Horace H. Rackham School of Graduate Studies of the University of Michigan.

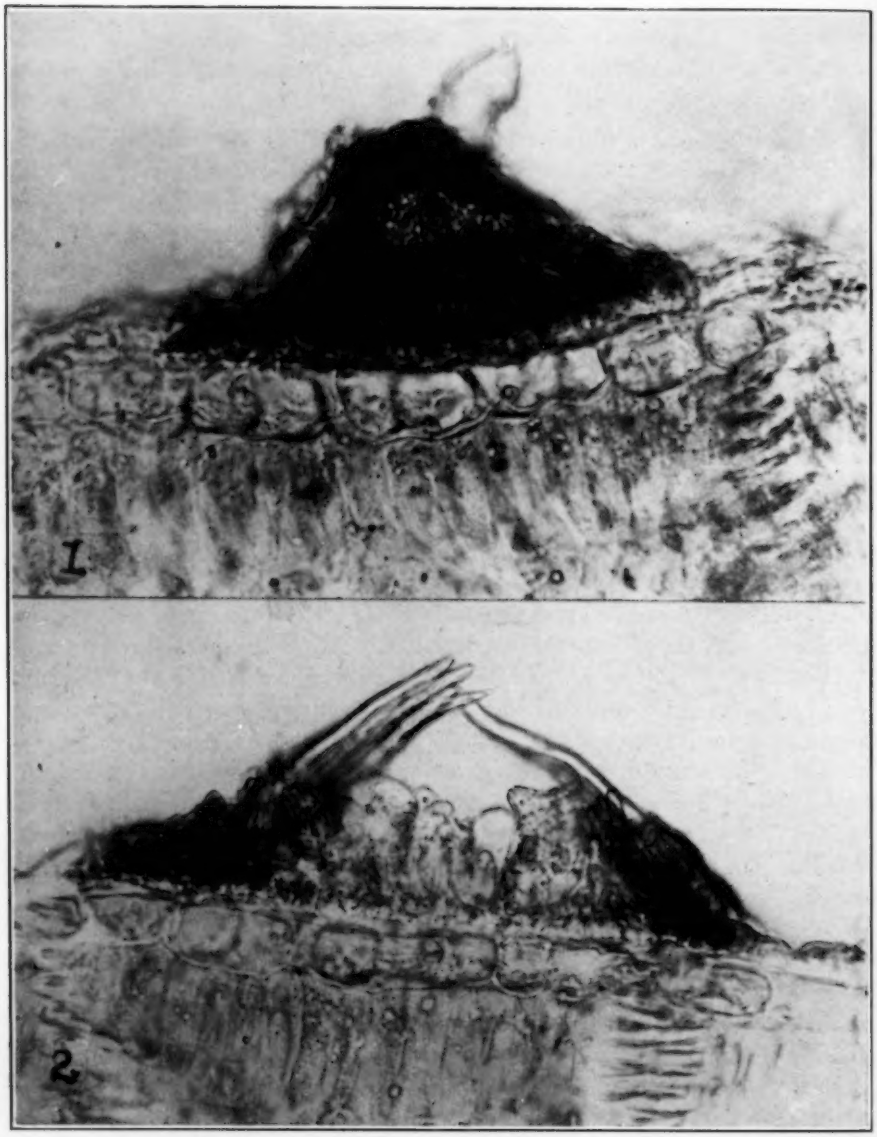


FIG. 1. Section through subcuticular pycnium, showing epidermal cells below, × 500.
FIG. 2. Section through subcuticular primary uredinium, showing overarching paraphyses, × 500.

and telia) are definitely subcuticular (figs. 1–3). The uredinia from the sporophytic mycelium are subepidermal (fig. 5). In cross section the sori from the gametophytic mycelium are triangular with broad bases.

Sydow (1937) has described a genus *Desmotelium* based on *D. coaetaneum*. He states that the pycnia, uredinia, and telia are all subcuticular. He places *Desmotelium* close to *Olivea* and *Chaconia*. The uredinia are described as bordered by paraphyses and the teliospores are given as one-celled, hyaline, and occurring in groups from basal cells. Through the kindness of H. Sydow, I have been able to study the type collection and find that all the sori are subepidermal. The cells of the epidermis of the host are thin-walled and are usually crushed by the developing sori. The uredinia are very broad with a flat hymenium bordered by paraphyses.

The collection from British Honduras apparently belongs in a new genus for which the following name is proposed.

Tegillum Mains, gen. nov. Pycnia subcuticularia; prima uredinia subcuticularia, paraphysibus ad bases coalitis cincta; urediniosporae echinulatae, angulares; secunda uredinia subepidermalia; teliosporae hyalinae, membranis tenuissimis, plures ex cellula conjunctim enatae, statim germinantes.

Species typica: *Tegillum fimbriatum*.

Tegillum fimbriatum Mains, sp. nov. (figs. 1–7). Pycnia subcuticularia, amphigena, aggregata in maculis magnis, denique brunneis, late conica, 90–120 × 40–60 μ.

Prima uredinia subcuticularia, late conica, plerumque epiphylla, aggregata cum pycniis, minuta, 0.1–0.3 mm. lata, paraphysibus cincta; paraphyses cylindraceae, ad bases coalitae, membranis intense brunneis, 1.5–2 μ latis; urediniosporae turbinatae, a latere compressae, 24–30 × 20–32 μ, membranis cinnamoneis, 1 μ latis, echinulatis, poris ad angulas sitae; secunda uredinia sparsa, hypophylla, 60–100 μ lata, subepidermalia, paraphysibus pallidis.

Telia plerumque hypophylla, eadem forma et distributione quam uredinia; teliosporae clavatae vel fusoideo-clavatae, 8–10 × 30–36 μ, membranis hyalinis, 0.5 μ latis, plures ex cellula conjunctim enatae, statim germinantes.

In foliis *Viticis* sp. (*Gaumeri* ?), San Agustin, El Cayo, British Honduras, VII, 24, 1936, E. B. Mains (*3881*, specimen typicum).

Pycnia subcuticular (fig. 1), amphigenous, grouped in large spots which finally become brown, broadly conical, 90–120 × 40–60 μ.

Primary uredinia subcuticular (fig. 2), broadly conical, mostly epiphyllous, grouped in large spots with the pycnia, small, 0.1–0.3 mm., bordered and mostly covered by the paraphyses except for an irregular central opening; paraphyses cylindric, often narrowing upward, with walls dark brown, 1.5–2 μ thick coalescing at their bases to form a short pseudo-peridium (fig. 4); urediniospores asymmetrical (figs. 6, 7), turbinate but laterally compressed and therefore broadly triangular, 24–30 × 20–32 μ, from one view, the upper side sometimes protruding upward into a fourth angle, ellipsoid or obovoid, 10–18 μ wide from the other view, with the walls 1 μ thick, cinnamon-brown, finely echinulate, with the pores in the angles; secondary

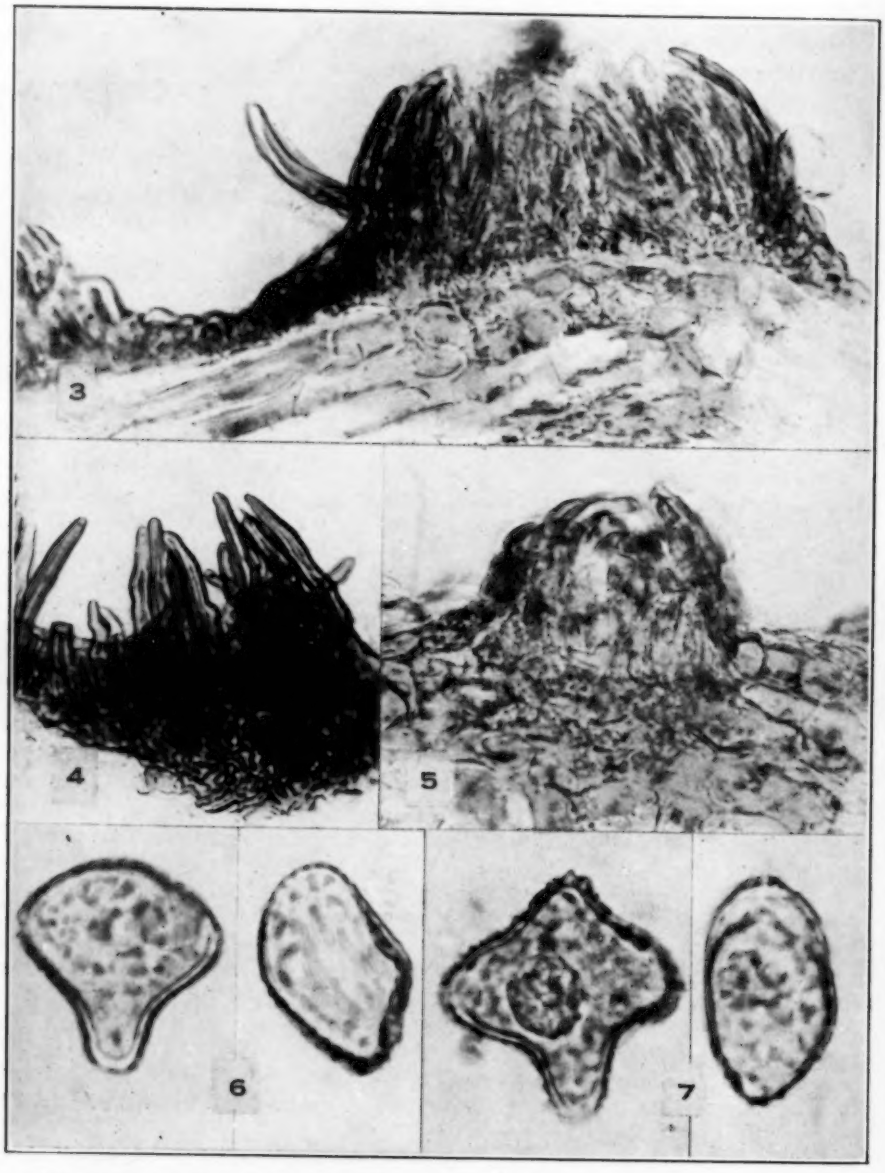


FIG. 3. Section through telium, × 500.
FIG. 4. Paraphyses from a membranous base, × 500.
FIG. 5. Subepidermal secondary uredinium, × 500.
FIG. 6. Urediniospore from two views, rotated slightly less than 90 degrees, × 1000.
FIG. 7. Urediniospore from two views, rotated 90 degrees, × 1000.

uredinia subepidermal (fig. 5), very small, 60–100 μ wide, hypophyllous, scattered mostly along margins of the leaves; paraphyses light colored, cylindric, incurved; urediniospores like those of the primary uredinia.

Telia mostly hypophyllous (fig. 3), grouped in spots with the pycnia and primary uredinia, similar to the primary uredinia; teliospores one-celled, clavate or fusoid-clavate, 8–10 × 30–36 μ, with the walls hyaline, very thin, 0.5 μ, occurring in groups (7 or more) from laterally free basal cells, germinating immediately.

On leaves of *Vitex* (probably *Gaumeri*) Gracie Rock, Sibun River, British Honduras Aug. 10, 1935, Percy H. Gentle (1756); San Agustin, El Cayo, British Honduras, E. B. Mains, July 23, 1936 (3775), July 24, 1936 (*3881*, type), Aug. 4, 1936 (4058).

The gametophytic mycelium forms a large spot, apparently gradually spreading outward from the original infection. As the infection ages, the spots turn brown. Pycnia are produced at the centers of the spots, and are followed by uredinia. Teliospores probably replace urediniospores in some of the uredinia, but as the infection ages telia are produced. The uredinia are bordered by paraphyses which slope inward and upward, covering the sori except for an irregular, central, pore-like opening. In section they appear triangular with their bases on the epidermal cells. The urediniospores are unusual in shape. Only a few secondary uredinia were seen. These are very small and subepidermal. All the telia seen were from gametophytic mycelia. Telia arising from the sporophytic mycelium will probably be subepidermal and resemble secondary uredinia.

Tegillum apparently belongs in the Oliveae of the Pucciniaceae of Dietel's classification (1928). The angular urediniospores, and hyaline, one-celled, clumped teliospores, both kinds developed in sori bordered by paraphyses, indicate a fairly close relationship to *Olivea* and *Desmotelium*. It differs from both in the position and development of sori from the gametophytic mycelium.

HERBARIUM OF THE UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN

Literature Cited

Arthur, J. C. 1917. Uredinales of Porto Rico based on collections by H. H. Whetzel and E. W. Olive. Mycologia **9**: 55–104.

———. 1925. Uredinales. N. Am. Flora **7**: 674.

Dietel, P. 1924. Kleine Beiträge zur Systematik der Uredineen. IV. Ann. Mycol. **22**: 269–273.

———. 1928. Uredinales. *In* Engler, A. & Prantl, K., Die natürlichen Pflanzenfamilien. **6**: 24–98.

Sydow, H. 1937. Novae fungorum species. XXV. Ann. Mycol. **35**: 244–286.

CHARACTERS FOR THE CLASSIFICATION AND IDENTIFICATION OF VARIETIES OF CAPSICUM[1]

H. L. COCHRAN

(WITH SIX FIGURES)

The Solanaceae contain several members on which studies have been made to determine the usefulness of various characters in the identification and classification of varieties. Salman (1924) used the leaf index for the identification of potato varieties and concluded that if certain precautions are used this character is constant for a given variety and remains so over a rather wide range of environment. Krantz and Hutchins (1928) also used the leaf index as a means of identifying certain American potato varieties and in their paper call attention to the limits of its usefulness. However, their final conclusions indicate that when varieties are grown under comparable conditions, the leaf index may be a valuable addition to the other characters which are utilized for the identification of varieties and the detection of synonyms. Lindstrom (1927) and Houghtaling (1935) have made use of the fruit-shape index in their studies on the inheritance of shapes of tomato fruits.

Although most botanists have come to recognize Bailey's (1924) classification of *Capsicum* which groups all the peppers in one species, namely *C. frutescens,* with five sub-species or varietal groups, there is still occasional confusion in properly classifying some horticultural varieties. It has been the chief aim of this paper, therefore, to bring together facts that may prove beneficial in the classification and identification of such varieties of *Capsicum.*

MATERIALS AND METHODS

Plants of 59 varieties of peppers were grown to maturity in 12-inch clay pots in the greenhouse during the winter of 1937 and in the field during the spring and summer of 1938. Observations and study were made of the leaves and fruits of both crops; definite measurements, however, were taken only on plants growing under field conditions. From 20 to 100 mature leaves and fruits were selected at random over the plots for each variety and their length and width accurately measured, the readings being taken to the nearest millimeter. The index for each variety was determined by the following formula: $\frac{W \times 100}{L}$. W represents the average width of the leaf for the variety, while L represents the average length. The width is multiplied by 100 in order to express the result as a percentage. The fruit-shape index

[1] Published with the approval of the Director as Journal Series paper number 68 of the Georgia Agricultural Experiment Station.

was computed for each fruit by dividing the length by the width. The probable error of the mean was calculated by the following formula: $PE_M = .8453\frac{\Sigma(+)d}{\sqrt{n(n-1)}}$. A typical leaf and fruit of each variety studied were outlined on paper for a record of their shape and size.

OBSERVATIONS AND DATA

From the data presented in table 1 it is seen that the leaf index varies over quite a wide range between varieties, the highest being 71.02 ± 0.68 for Sweet Meat Glory and the lowest 39.95 ± 0.27 for Minimum. As a whole, however, the index figures are higher for the mild-flavored varieties than for the pungent ones. Correspondingly in table 1, but possibly more clearly in figures 1, 2, and 3, it is to be noted that there exists an even more consistent difference between leaf sizes for the two varietal groups cited above. Almost uniformly large leaves are associated with mild-flavored varieties while small leaves are associated with pungent varieties. Further analysis of these data indicate the existence of a definite relationship between leaf size and fruit size for the varieties studied. The coefficient of correlation between leaf length and fruit length and between leaf width and fruit width was 0.364 and 0.733 respectively. Both of these values are highly significant. The correlation between leaf shape and fruit shape was 0.028.

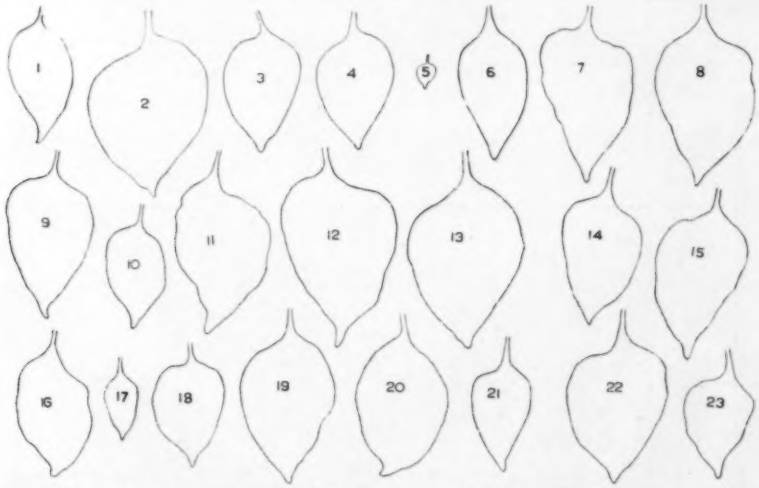


FIG. 1. Trace drawings of typical mature leaves of the varieties shown in table 1.

While no actual measurements were made of leaves from the plants of any variety growing in the greenhouse, observations indicated clearly that leaf sizes were larger under these conditions than those of the same variety growing under field conditions, the difference being due, no doubt, to a

TABLE 1

Leaf and fruit characters of 59 varieties of Capsicum

Variety number	Variety name	Average leaf length	Average leaf width	Leaf index	Average fruit length	Average fruit width	Fruit shape index	Fruit[1] flavor
		cm.	*cm.*		*cm.*	*cm.*		
1	Anheim Chili	8.84	4.51	51.02 ± 0.48	12.90	3.64	3.54 ± 0.06	P
2	Asgrow Calwonder	12.55	8.25	65.74 ± 0.58	10.03	8.43	1.19 ± 0.01	M
3	Asgrow King	9.01	5.67	62.93 ± 0.59	11.31	7.44	1.52 ± 0.04	M
4	Bell or Bull Nose	8.73	5.68	65.06 ± 0.56	5.98	6.78	0.88 ± 0.02	M
5	Bird's Eye	2.09	1.40	66.99 ± 0.60	0.94	0.67	1.40 ± 0.02	P
6	Burpee's Sunnybrook	9.96	5.01	50.30 ± 0.47	4.54	6.85	0.66 ± 0.01	M
7	California Wonder	11.61	6.89	59.35 ± 0.57	8.47	7.25	1.17 ± 0.03	M
8	California Wonder Bell	11.94	7.24	60.64 ± 0.48	7.75	8.11	0.69 ± 0.03	M
9	Cayenne	10.67	6.13	57.45 ± 0.52	11.39	1.43	7.97 ± 0.14	P
10	Celestial	7.77	4.26	54.83 ± 0.50	2.68	1.81	1.48 ± 0.02	P
11	Certified Florida	11.82	6.64	56.18 ± 0.54	8.23	8.10	1.02 ± 0.02	M
12	Certified Ruby King	13.00	8.31	63.92 ± 0.57	11.03	6.53	1.69 ± 0.03	M
13	Chinese Giant	12.95	8.56	66.10 ± 0.49	7.97	8.87	0.90 ± 0.06	M
14	Crimson Giant	9.75	5.95	61.03 ± 0.58	8.81	6.58	1.34 ± 0.03	M
15	Early Dwarf California Wonder	11.58	6.42	55.44 ± 0.39	8.75	7.85	1.11 ± 0.02	M
16	Early Normandie	9.80	5.25	53.57 ± 0.46	10.70	6.93	1.54 ± 0.04	M
17	Florida Gem	5.04	2.47	49.01 ± 0.40	4.58	2.79	1.64 ± 0.02	P
18	Golden Dawn	8.11	5.15	63.50 ± 0.57	8.90	5.84	1.52 ± 0.04	M
19	Giant King	11.26	6.93	61.55 ± 0.51	10.94	6.79	1.61 ± 0.05	M
20	Golden Queen	10.53	6.75	64.10 ± 0.55	9.57	7.60	1.26 ± 0.05	M
21	Green Mexican Pickling	8.09	4.39	54.26 ± 0.35	6.60	3.30	2.00 ± 0.04	P
22	Harris' Early Giant	11.05	7.37	66.70 ± 0.60	8.03	7.08	1.13 ± 0.02	M
23	Harris' Earliest	7.87	5.05	64.17 ± 0.55	8.29	5.83	1.42 ± 0.04	M
24	Harris' Wonder	10.54	7.20	68.31 ± 0.42	10.88	7.19	1.51 ± 0.04	M
25	Harris' King of the North	9.57	5.46	57.05 ± 0.39	7.68	7.17	1.07 ± 0.03	M
26	Harris' Improved Squash	11.01	6.28	57.04 ± 0.59	4.77	6.68	0.71 ± 0.01	M
27	Holmes' Prolific Sweet	9.56	6.05	63.28 ± 0.62	9.01	7.40	1.22 ± 0.03	M
28	Hot Portugal	8.32	4.57	54.93 ± 0.54	15.07	3.05	4.94 ± 0.01	P
29	Hungarian Wax	8.68	5.04	58.06 ± 0.50	13.61	3.54	3.84 ± 0.01	P
30	Hungarian Yellow	8.80	4.21	47.84 ± 0.41	12.93	3.55	3.64 ± 0.01	P

[1] M = Mild; P = Pungent.

[1] M = Mild; P = Pungent.

TABLE 1—(*Continued*)

Variety number	Variety name	Average leaf length	Average leaf width	Leaf index	Average fruit length	Average fruit width	Fruit shape index	Fruit[1] flavor
		cm.	*cm.*		*cm.*	*cm.*		
31	Improved Collossal	12.65	7.53	59.53 ± 0.53	10.32	7.42	1.39 ± 0.03	M
32	Leonard's Early Wonder	10.59	5.12	48.35 ± 0.46	9.83	5.48	1.79 ± 0.01	M
33	Little Gem	4.74	2.39	50.42 ± 0.34	3.07	1.92	1.60 ± 0.02	P
34	Long Red Cayenne	9.03	4.87	53.93 ± 0.48	11.89	2.58	4.61 ± 0.09	P
35	Low Bush California Wonder	10.59	6.54	61.76 ± 0.52	8.17	7.62	1.07 ± 0.04	M
36	Long Thick Red	8.07	3.81	47.21 ± 0.39	8.83	3.17	2.79 ± 0.09	P
37	Meales Red Hot	11.40	5.48	48.07 ± 0.47	14.04	3.30	4.25 ± 0.06	P
38	Mexican Chili	8.62	5.05	58.58 ± 0.51	8.19	4.81	1.70 ± 0.02	P
39	Minimum	3.93	1.57	39.95 ± 0.27	1.76	1.06	1.66 ± 0.03	P
40	New Mexico No. 9	9.41	5.24	55.69 ± 0.41	12.95	3.44	3.76 ± 0.05	P
41	Neapolitan	7.50	4.26	56.80 ± 0.50	10.79	4.82	2.24 ± 0.06	M
42	Oshkosh	8.55	4.72	55.20 ± 0.52	6.71	7.00	0.96 ± 0.02	M
43	Paul's Jersey Giant	8.31	4.90	58.97 ± 0.63	9.08	8.22	1.10 ± 0.04	M
44	Perfection Pimiento	12.00	8.05	67.08 ± 0.57	7.18	6.32	1.14 ± 0.02	M
45	Red Chili	8.94	5.67	63.42 ± 0.52	12.65	3.35	3.78 ± 0.08	P
46	Red Oshkosh	11.56	6.42	55.54 ± 0.49	7.06	7.90	0.89 ± 0.03	M
47	Rocky Ford	10.28	5.35	52.04 ± 0.55	10.15	8.02	1.27 ± 0.04	M
48	Ruby Giant	11.37	7.42	65.26 ± 0.60	8.73	8.28	1.05 ± 0.02	M
49	Ruby King	12.75	7.45	58.43 ± 0.57	11.03	7.20	1.53 ± 0.02	M
50	Simon's World Beater	13.19	7.29	55.27 ± 0.46	10.35	7.62	1.36 ± 0.02	M
51	Squash or Tomato	10.07	6.40	63.56 ± 0.44	4.20	6.30	0.67 ± 0.01	M
52	Sport	7.24	4.41	60.91 ± 0.53	10.73	1.47	7.30 ± 0.02	P
53	Sweet Meat Glory	12.32	8.75	71.02 ± 0.68	8.88	6.46	1.37 ± 0.06	M
54	Sweet Yellow	12.21	7.24	59.30 ± 0.49	7.40	6.48	1.14 ± 0.02	M
55	Tabasco	9.05	4.61	50.94 ± 0.56	3.01	0.54	5.57 ± 0.12	P
56	Talinette	9.07	4.77	52.59 ± 0.54	14.88	4.22	3.53 ± 0.13	P
57	Waltham Beauty	11.04	6.93	62.77 ± 0.57	7.75	7.95	0.97 ± 0.03	M
58	Windsor A	9.55	6.34	66.39 ± 0.60	10.54	6.54	1.61 ± 0.03	M
59	Windsor B	8.52	4.68	54.93 ± 0.51	9.92	7.14	1.39 ± 0.02	M

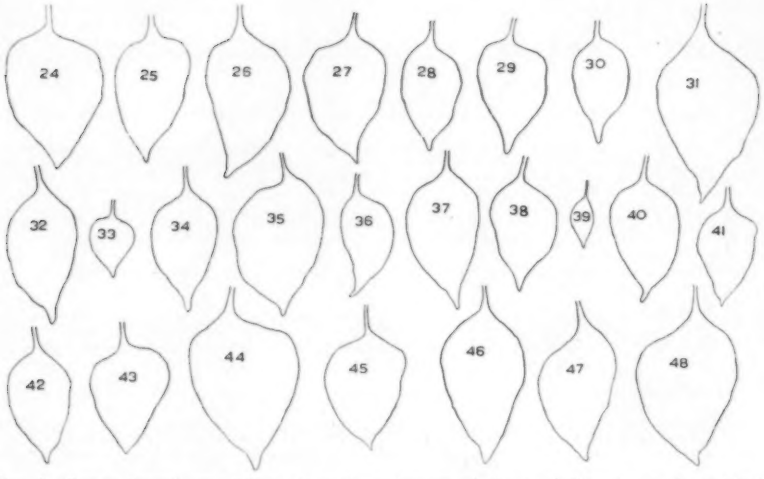


FIG. 2. Trace drawings of typical mature leaves of the varieties shown in table 1.

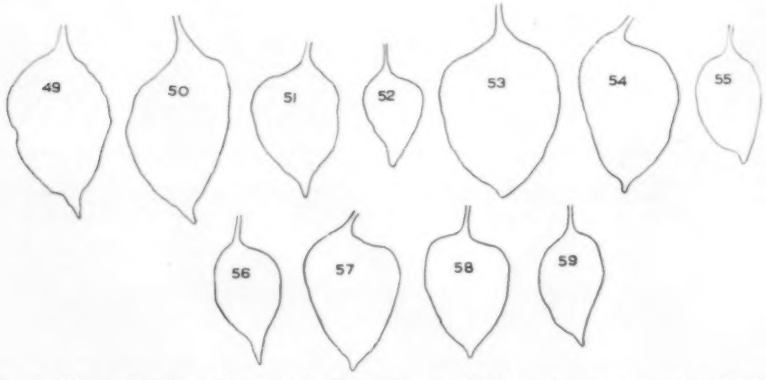


FIG. 3. Trace drawings of typical mature leaves of the varieties shown in table 1.

better growing environment in the former, and especially to moisture. Had the leaf index been computed for the varieties growing in the greenhouse, the figures obtained would have differed to some extent from those of the same variety growing in the field, perhaps as much as between some varieties. This observation is substantiated by the findings of Krantz and Hutchins (1928), who grew potatoes in several locations in the State of Minnesota and found that the environment produced significant differences in the leaf index of all varieties tested except one, which remained constant.

While measurements were being made of mature leaves from the field plots, it was rather surprising to learn of the uniformity of size among mature leaves within a given variety. Apparently the leaf index should remain rather constant for a given variety under comparable conditions.

By comparing the data in table 1, under the fruit-shape index column, with the fruit tracings of the varieties in figures 4, 5, and 6, it is evident

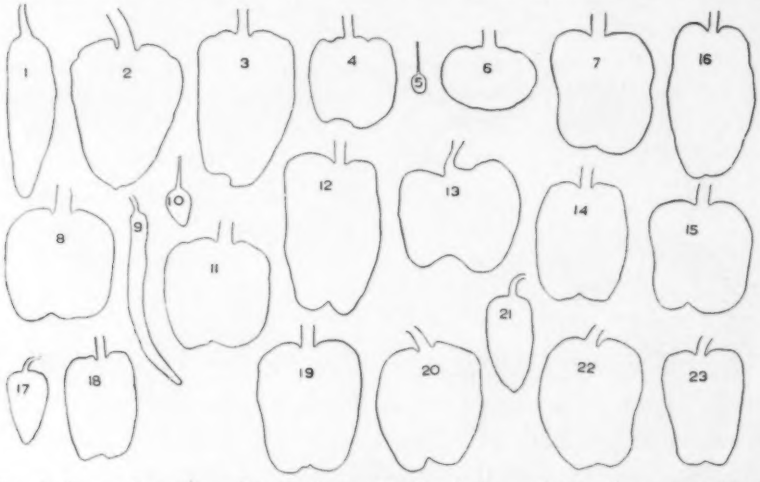


FIG. 4. Trace drawings of typical ripe fruits of the varieties shown in table 1.

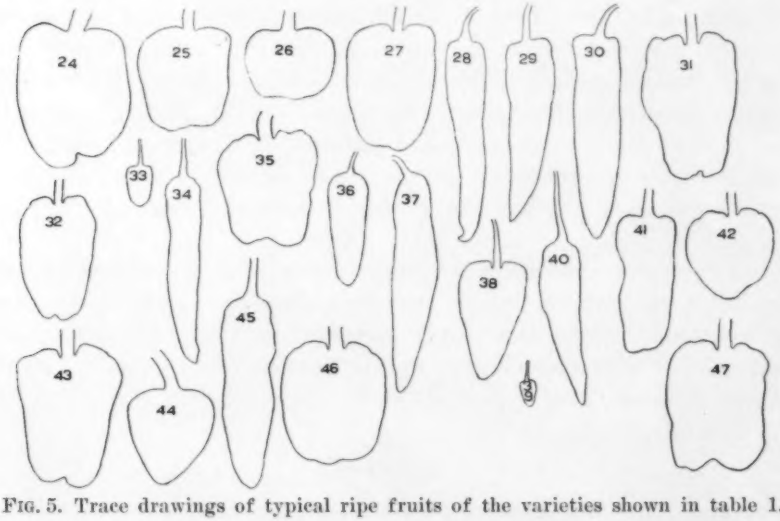


FIG. 5. Trace drawings of typical ripe fruits of the varieties shown in table 1.

that varieties may be grouped into two general classes according to fruit shape. The first group contains varieties with from blocky to almost spherical fruits having shape indices ranging from 0.66 ± 0.01 to 2.24 ± 0.06, while the second group contains long fruits of various shapes with indices ranging from 1.40 ± 0.02 to 7.97 ± 0.14. Previous observations by the writer, as well as definite measurements made during this study, show that in some varieties the shape of the ovary changes greatly between the stages

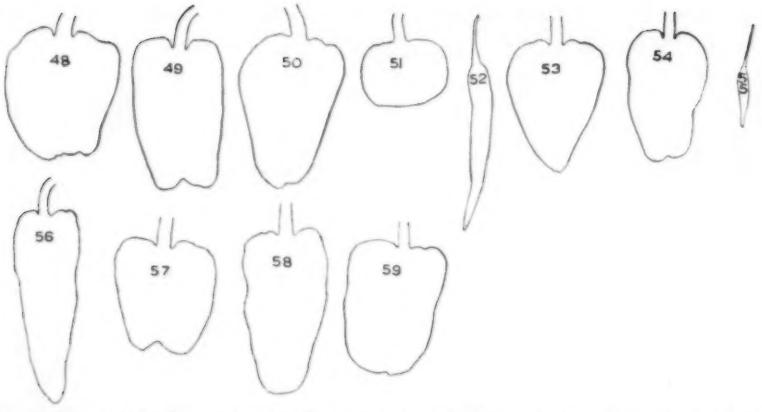


FIG. 6. Trace drawings of typical ripe fruits of the varieties shown in table 1.

of anthesis and fruit maturity. These findings agree fully with those reported earlier by Sinnott and Kaiser (1934), who showed that most shape types in *Capsicum* have fruit primordia that are essentially isodiametric at the time of flowering, but that this relation is maintained only in the spherical types until maturity. In the elongated fruit type the authors state further that a much more rapid growth takes place in length than in width. By far the most complete analysis of the above-stated change in fruit shapes in *Capsicum* has been that reported by Kaiser (1935). From his work it is evident that environmental conditions influence fruit shape to some extent but the ultimate configuration of the fruits depends on the interaction between genes which control shape development and those which govern size.

When the same varieties were grown under both greenhouse and field conditions, it was quite noticeable that fruit shapes are more easily altered by environment than are leaf shapes. Nevertheless, under the conditions of this experiment both characters proved to be relatively constant. This does not mean, however, that either could be used alone in making positive variety identifications.

SUMMARY

Leaf and fruit measurements were made and their respective indices computed for 59 varieties of *Capsicum* grown under field conditions. Observations were made on the same varieties growing in the greenhouse. It was found that there exists a definite relationship between leaf size and fruit size but little correlation between leaf shape and fruit shape. The index figures extend over a rather wide range among varieties, as would be expected. However, the leaf and fruit index may be valuable together with certain other plant characters in the classification and identification of

pepper varieties grown under comparable conditions, but would seemingly be of little value when used alone.

The writer is indebted to Mr. Leslie R. Hawthorn of Texas Substation No. 19 at Winter Haven, Texas; to Dr. Roy Magruder of the U. S. Horticultural Station at Beltsville, Maryland; and to Dr. Paul Work of Cornell University at Ithaca, New York, for furnishing the seeds of some of the pepper varieties used in this study.

Department of Horticulture,
Georgia Agriculture Experiment Station
Experiment, Georgia

Literature Cited

Bailey, L. H. 1924. Manual of cultivated plants. The MacMillan Company, New York.

Houghtaling, Helen B. 1935. A developmental analysis of size and shape in tomato fruits. Bull. Torrey Club **62:** 243–252.

Kaiser, Samuel. 1935. The factors governing shape and size in Capsicum fruits; a genetic and developmental analysis. Bull. Torrey Club **62:** 433–454.

Krantz, F. A. & Hutchins, A. E. 1928. The "Leaf Index" of some American potato varieties. Proc. 15th annual meeting Potato Assoc. Am.

Lindstrom, E. W. 1927. The inheritance of ovate and related shapes of tomato fruits. Jour. Agr. Res. **34:** 961–985.

Salman, R. N. 1924. A leaf index as a help to the identification of potato varieties. Proc. Camb. Phil. Soc. Biol. **1:** 121.

Sinnott, E. W. & Kaiser, S. 1934. Two types of genetic control over the development of shape. Bull. Torrey Club **61:** 1–7.

INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

This Index is reprinted monthly on cards, and furnished in this form to subscribers at the rate of three cents for each card. Selections of cards are not permitted; each subscriber must take all cards published during the term of his subscription. Correspondence relating to the card issue should be addressed to the Treasurer of the Torrey Botanical Club.

Albrecht, W. A. & Schroeder, R. A. Collodial clay culture for refined control of nutritional experiments with vegetables. Proc. Am. Soc. Hort. Sci. **37:** 689–692. My 1940.

Alexander, E. J. *Aechmea fulgens discolor.* Native of Brazil. Addisonia **21:** 17, 18. *pl. 681.* 6 Au 1940.

Alexander, E. J. *Penstemon dissectus.* Native of southern Georgia. Addisonia **21:** 23, 24. *pl. 684.* 6 Au 1940.

Alexander, E. J. *Rondeletia leucophylla.* Native of Mexico. Addisonia **21:** 31. *pl. 688.* 6 Au 1940.

Alexander, E. J. *Sanchezia parvibracteata.* Native of tropical America? Addisonia **21:** 21, 22. *pl. 683.* 6 Au 1940.

Alexander, E. J. *Solanum sisymbrifolium.* Native of tropical America. Addisonia **21:** 27, 28. *pl. 686.* 6 Au 1940.

Allard, H. A. A globose form of pitch pine (*Pinus rigida*) in Virginia. Rhodora **42:** 308. 13 Au 1940.

Allard, H. A. & Allard H. F. The wild bean *Phaseolus polystachyus* (L.) B. S. P.: Its chromosome number. Jour. Wash. Acad. Sci. **30:** 335–337. 15 Au 1940.

Alphin, T. H. A descriptive study of varietal forms in *Buxus.* Am. Jour. Bot. **27:** 349–357. *pl. 1, 2 + f. 1.* 6 Jl 1940.

Alston, A. H. G. The *Selaginellae* of Argentina, Uruguay and Paraguay. Physis **15:** 251–257. 31 Mr 1939.

Anderson, W. A. A little-known collection from Missouri. Rhodora **42:** 280. 13 Au 1940.

Alexrod, D. I. The Mint Canyon flora of southern California: a preliminary statement. Am. Jour. Sci. **238:** 577–585. Au 1940.

Baez, J. B. Dos aspectos de la vegetación del norte de San Luis. Physis **15:** 357–376. *f. 1–11.* 31 Mr 1939.

Baker, M. S. Studies in western violets—III. Madroño **5:** 218–231. *pl. 20–23.* 10 Jl 1940.

Balme, J. Algunos datos sobre Orquideas Colombianas. Rev. Acad. Colombiana **3:** 345, 346. *2 pl.* Mr–Ap 1940.

Bandoni, A. J. & O'Donell, C. A. La anatomie de la timeleácea *Ovidia pillo-pillo.* Planta medicinal de los Andes patagónicos. Physis **15:** 377–385. *f. 1–5.* 31 Mr 1939.

Bartram, E. B. Mosses of southeastern Polynesia. Bishop Mus. Occ. Pap. **15:** 324–349. *f. 1–12.* 29 My 1940.

Bartram, E. B. Mosses of Trans-Pecos Texas. Bryologist **43:** 95–98. *f. 1–10.* Au 1940.

Batalla, M. A. Estudio morfológico de los granos de polen de las plantas vulgares del valle de México. An. Inst. Biol. Mexico **11:** 129–161. *f. 1–87.* 1940.

Beath, O. A., Gilbert, C. S. & Eppson, H. F. The use of indicator plants in locating seleniferous areas in western United States. III. Further studies. Am. Jour. Bot. **27:** 564–573. *f. 1–8.* Au 1940.

Beaudoin, O. *Impatiens parviflora* DC. à Chateauguay-Bassin, P. Q. Ann. Acfas **6:** 108. 1940.

Bishop, H. A study of sexuality in *Sapromyces Reinschii.* Mycologia **32:** 505–529. *f. 1–6.* Jl–Au 1940.

Blomquist, H. L. Another new species of *Plagiochila* from the southern Appalachian Mountains. Bryologist **43:** 89–95. *f. 1–12.* Au 1940.

Boivin, B. Quelques nouvelles espèces de plantes vasculaires introduites ou indigènes pour le Québec. Ann. Acfas **6:** 108. 1940.

Bowden, W. M. Diploidy, polyploidy, and winter hardiness relationships in the flowering plants. Am. Jour. Bot. **27:** 357–371. *pl. 1.* 6 Jl 1940.

Boyce, J. S. A needle-cast of Douglas fir associated with *Adelopus gäumanni.* Phytopathology **30:** 649–659. *f. 1, 2.* Au 1940.

Brenckle, J. F. & Cottam, W. P. A new *Polygonum* from Garfield County, Utah. Bull. Univ. Utah **30**[15]: 1–7. *pl. 1.* 27 Je 1940.

Broyer, T. C. & Hoagland, D. R. Methods of sap expression from plant tissues with special reference to studies on salt accumulation by excised barley roots. Am. Jour. Bot. **27:** 501–511. *f. 1–5.* Au 1940.

Broyer, T. C. & Overstreet, R. Cation exchange in plant roots in relation to metabolic factors. Am. Jour. Bot. **27:** 423–430. *f. 1–4.* 6 Jl 1940.

Brown, J. R. *Sedum Palmeri* and *Sedum compressum.* Cactus & Succulent Jour. **12:** 97–100. *illust.* Je 1940.

Brown, R. W. New species and changes of name in some American fossil floras. Jour. Wash. Acad. Sci. **30:** 344–356. *f. 1–18.* 15 Au 1940.

Brunel, J. Addition du *Micrasterias expansa* à la flore du Québec. Ann Acfas **6:** 104. 1940.

Bukasov, S. M. Interspecific hybridization in the potato. Physis **18:** 269–284. 31 My 1939.

Burkart, A. Frecuencia de la fecundación cruzada en la alfalfa según experimencias con plantas recesivas de flor blanca y consideraciones sobre el mejoramiento de esta forrajera. Physis **18:** 365, 366. 31 My 1939.

Burkart, A. Las especies argentinas del genero *Centrosema* (Leguminosae). Physis **15:** 301–304. 31 Mr 1939.

Burkart, A. Materiales para una monografia del genero *Prosopis* (Leguminosae). Darwiniana **4:** 57–128, *pl. 1–23* + *f. 1–15,* 25 Je 1940.

Burkart, A. Nota sobre algunas leguminosas indigenas o introducidas en Chile. Rev. Chilena Hist. Nat. **43:** 156–164. 1939.

Burkart, A. Nota sobre plantas adventicias de la Flora Argentina. Darwiniana **4:** 141–144. 25 Je 1940.

Burkholder, P. R. & McVeigh, I. Growth and differentiation of maize in relation to nitrogen supply. Am. Jour. Bot. **27:** 414–424. *f.* 1–10. 6 Jl 1940.

Cabrera, A. L. Cuatro compuestas nuevas de la América Austral. Darwiniana **4:** 133–140. *f. 1–4.* 25 Je 1940.

Cabrera, A. L. Las Compuestas mendocinas de Philippi. Physis **15:** 291–299. 31 Mr 1939.

Campbell, D. H. The evolution of the land plants [Embryophyta]. i–ix, 1–731. *f. 1–351.* Stanford University Press, Stanford University, California, 1940.

Carabia, J. P. Estudio bio-geográfico de la zona de Pilón. Rev. Soc. Geografica Cuba **12:** 67–84. *f. 1–7.* 1939.

Carlson, G. G. & Jones, V. H. Some notes on uses of plants by the Comanche Indians. Papers Mich. Acad. Sci. **25:** 517–542. 1940.

Carrera, C. J. M. El genero *Fusarium* en la Republica Argentina. Estudio e identificación de algunas especies. Physis **15:** 21–77. *f. 1–24.* 31 Mr 1939.

Cash, E. K. A second note on Georgia Discomycetes. Jour. Wash. Acad. Sci. **30:** 299–305. *f. 1.* 15 Jl 1940.

Cendrero, O. C. Curso elemental de historia natural. Botánica. 7th ed. 1–295 p. *f. 1–718.* Paris, 1938.

Charles, V. K. An entomogenous fungus on spider mites on water hyacinth. Mycologia **32:** 537–540. Jl–Au 1940.

Chilton, S. P. Delayed reduction of the diploid nucleus in promycelia of *Ustilago zeae.* Phytopathology **30:** 622, 623, *f. 1.* Jl 1940.

Christensen, J. J. & Rodenhiser, H. A. Physiologic specialization and genetics of the smut fungi. Bot. Rev. **6:** 389–425. Au 1940.

Clark, F. J. Cytogenetic studies of divergent meiotic spindle formation in *Zea Mays.* Am. Jour. Bot. **27:** 547–559. *pl. 1–3* + *f. 1–3.* Au 1940.

Clements, H. F. & Akamine, E. Root stimulation in sugar cane (*Saccharum officinarum*) with special reference to the effects of ethyl alcohol. Am. Jour. Bot. **27:** 482–487. *pl. 1.* Au 1940.

Clos, E. C. Los tipos de mani (*Arachis hypogaea*) cultivados en la Argentina y su distribición geográfica. Physis **18:** 317–329. *f. 1–12.* 31 My 1939.

Contardi, H. G. Estudios genéticos en *Cucurbita* y consideraciones agronómicas. Physis **18:** 331–347. *f. 1–6.* 31 My 1939.

Cook, O. F. Aublet the botanist, a pioneer against slavery, with a memorial genus of palms. Jour. Wash. Acad. Sci. **30:** 294–299. *f. 1.* 15 Jl 1940.

Copeland, H. F. The phylogeny of the Angiosperms. Madroño **5:** 209–218. *f. 1–2.* 10 Jl 1940.

Core, E. L. The flora of Roaring Plains, West Virginia. Proc. W. Va. Acad. Sci. **12:** 33–35. 1939.

Core, E. L. Notes on the Mid-Appalachian species of *Paronychia.* Virginia Jour. Sci. **1:** 110–116. My 1940.

Cormack, M. W. *Phytophthora cactorum* as a cause of root rot in sweetclover. Phytopathology **30:** 700, 701. Au 1940.

Cottam, W. P. A new violet from Utah. Bull. Univ. Utah **29**[13]: 1–7. *pl. 1.* 28 Je 1939.

Cottam, W. P., Garrett, A. O. & Harrison, B. F. New and extended range for Utah plants. Bull. Univ. Utah **30**[16]: 1–11. 29 Je 1940.

Cotter, R. U. Unseasonable germination of teliospores of *Puccinia graminis tritici.* Phytopathology **30:** 689–691. Au 1940.

Cotter, R. U. An unusual telial collection of *Puccinia graminis.* Phytopathology **30:** 693–695. Au 1940.

Crane, M. B. The origin of new forms in *Rubus.* II. The loganberry, *R. loganbaccus* Bailey. Jour. Genet. **40:** 129–139. *pl. 5* + *f. 1.* My 1940.

Crane, M. B. Reproductive versatility in *Rubus.* I. Morphology and inheritance. Jour. Genet. **40:** 109–118. My 1940.

Croizat, L. Notes on the Dilleniaceae and their allies: *Austrobaileyeae* subfam. nov. Jour. Arnold Arb. **21:** 397–404. Jl 1940.

Cross, G. L. Development of the foliage leaves of *Taxodium distichum*. Am. Jour. Bot. **27**: 471–482. *pl. 1, 2+f. 1–24*. Au 1940.

Daniel, H. Anotaciones marginales a la "Monografia de las Leguminosas," de Santiago Cortes. Rev. Acad. Colombiana **3**: 327–331. Mr–Ap 1940.

Davy, J. B. The botanical name of the Nargusta. Tropical Woods **63**: 38. 1 S 1940.

Deam, C. C. Flora of Indiana. 1–1236 p. *illust*. Dept. Conservation, Div. Forestry, Indianapolis, 1940.

De Fina, A. L. Sumas de temperaturas y Duraciones del dia que determinan la floración del lino (*Linum usitatissimum* L.). Physis **18**: 291–315. 31 My 1939.

Devoto, F. E. Las hibridaciones entre especies forestales y sus frecuentes hibridaciones naturales en nuestro pais. Physis **18**: 369–374. *f. 1–4*. 31 My 1939.

Dowding, E. S. & Buller, A. H. R. Nuclear migration in *Gelasinospora*. Mycologia **32**: 471–488. *f. 1–6*. Jl–Au 1940.

Drechsler, C. Three new Hyphomycetes preying on free-living terricolous nematodes. Mycologia **32**: 448–470. *f. 1–3*. Jl–Au 1940.

Dugand, A. La botánica sistematica en Colombia. Rev. Fac. Nac. Agron. **2**: 368–376. Ja–Ap 1940.

Dunkle, M. B. Contributions from the Los Angeles Museum—Channel Islands Biological Survey. 7. A revision of the Channel Island forms of *Cercocarpus*. Bull. So. Calif. Acad. Sci. **39**: 1, 2. Ja–Ap 1940.

Dunkle, M. B. Contributions from the Los Angeles Museum—Channel Islands Biological Survey. 8. Ferns of the Channel Islands. Bull. So. Calif. Acad. Sci. **39**: 2–5. Ja–Ap 1940.

Eastwood, A. New western plants. Leafl. West. Bot. **2**: 263–269. 18 Jl 1940.

Ehrlich, J. & Opie, R. S. Mycelial extent beyond blister rust cankers on *Pinus monticola*. Phytopathology **30**: 611–620. *f. 1*. Jl 1940.

Eigsti, O. J. The effects of colchicine upon the division of the generative cell in *Polygonatum*, *Tradescantia*, and *Lilium*. Am. Jour. Bot. **27**: 512–524. *f. 1–66*. Au 1940.

Elrod, R. P. & Snell, W. H. Development of the carpophores of certain Boletaceae. Mycologia **32**: 493–504. *f. 1, 2*. Jl–Au 1940.

Everett, T. H. *Glycosmis citrifolia*. Native of tropical America. Addisonia **21**: 29, 30. *pl. 687*. 6 Au 1940.

Everett, T. H. *Primula obconica*. Native of China. Addisonia **21**: 25, 26. *pl. 685*. 6 Au 1940.

Everett, T. H. *Tradescantia Warszewicziana*. Native of Central America. Addisonia **21**: 19, 20. *pl. 682*. 6 Au 1940.

Farwell, O. A. Contributions to the botany of Michigan No. 17. Rhodora **42**: 306–308. 13 Au 1940.

Fassett, N. C. A manual of aquatic plants. i–vii, 1–382 p. *illust*. McGraw Hill, New York, 1940.

Faura, R. E. Datos sobre *Cassia leptocarpa* Bentham. Physis **18**: 430–440. *f. 1–3*. 31 My 1939.

Faura, R. E. Estudio fitoquimico de la *Flourensia riparia* Grisebach. Physis **18**: 425–432. *f. 1, 2*. 31 My 1939.

Fernald, M. L. Some Spermatophytes of eastern North America. Rhodora **42**: 239–276. *pl. 598–605*. 15 Jl 1940; 281–302. *pl. 606–625*. 13 Au 1940.

Floriani, L. Examen fitoquimico de la liga de Mendoza [*Psittacanthus cuneifolius* (Ruiz et Pavon) Engler]. Physis **18**: 441–443. 31 My 1939.

Fosberg, F. R. Notes on Micronesian Rubiaceae. Bishop Mus. Occ. Pap. **15:** 213–226. 10 Ja 1940.

Foster, A. S. Further studies on zonal structure and growth of the shoot apex of *Cycas revoluta* Thunb. Am. Jour. Bot. **27:** 487–501. *f. 1–12.* Au 1940.

Frampton, V. L. The thixotropic character of the tobacco-mosaic virus protein. Phytopathology **30:** 666–673. *f. 1–3.* Au 1940.

Frezzi, M. J. La prescencia del *Fusarium bulbigenum* v. *blasticola* en le Republic Argentina. Physis **15:** 87–97. *f. 1–6.* 31 My 1939.

Gagnon, A., Corrivault, G. W. & Morin, A. Notes sur la flore saguenayenne. Ann. Acfas **6:** 107. 1940.

Gauthier, R. & Boivin, B. La *Vaccinium ovalifolium* dans le Québec. Ann. Acfas **6:** 107, 108. 1940.

Gazet du Chatelier, S. Récherches sur les Sterculiacées. I. Introduction. Matériel d'etudes. Rev. Gen. Bot. **52:** 174–182. 1940.

Giardelli, M. L. Nuevas especies de Lemnáceas para la Flora Argentina. Physis **15:** 323–329. *f. 1, 2.* 31 Mr 1939.

Gillette, N. J. Some Miocene plants from north central Idaho. Northwest Sci. **14:** 51–55. *pl. 1 + f. 1.* Au 1940.

Glover, S. L. Pleistocene deformation in the Olympic Coastal region. Northwest Sci. **14:** 69–71. Au 1940.

Grace, N. H. Effects of dust containing indolebutyric acid and oestrone on the rooting of dormant *Lonicera tartarica* cuttings. Canad. Jour. Res. **18:** 283–288. Jl 1940.

Groh, H. & Senn, H. A. *Prunus* in eastern Canada. Canad. Jour. Res. **18:** 318–346. *f. 1–10.* Jl 1940.

Gross, E. W. The responses of shoots of mungo bean seedlings growing in solutions of 3-indole-acetic acid. Am. Jour. Bot. **27:** 371–376. *f. 1–5.* 6 Jl 1940.

Grout, A. J. Mosses in Florida lime-sinks. Bryologist **43:** 112. Au 1940.

Hall, T. F. The biology of *Saururus cernuus* L. Am. Midl. Nat. **24:** 253–260. *f. 1–3.* Jl 1940.

Hansen, H. P. Paleoecology of a montane peat deposit at Bonaparte Lake, Washington. Northwest Sci. **14:** 60–68. *f. 1.* Au 1940.

Hare, J. F. & King, C. J. The winter carry-over of angular leaf spot infection in Arizona cotton fields. Phytopathology **30:** 679–684. *f. 1, 2.* Au 1940.

Harris, H. A. Comparative wilt induction by *Erwinia tracheiphila* and *Phytomonas Stewarti.* Phytopathology **30:** 625–638. Au 1940.

Heimsch, C. Wood anatomy and pollen morphology of *Rhus* and allied genera. Jour. Arnold Arb. **21:** 279–291. *pl. 1–3.* Jl 1940.

Hermann, F. J. The geographic distribution of *Pilea fontana.* Torreya **40:** 118–120. Jl–Au 1940.

Herre, A. W. C. T. A new species of *Lecidea* from Brazil. Madroño **5:** 235, 236. 10 Jl 1940.

Hershey, A. L. Notes on plants of New Mexico—II. Leafl. West. Bot. **2:** 257, 258. 18 Jl 1940.

Hirschhorn, E. Refundición del genero *Sphacelothera* en *Ustilago.* Physis **15:** 103–111. 31 Mr 1939.

Hirschhorn, E. Suplemento a las especies de *Cintractia* de la flore Argentina. Rev. Argentina Agron. **7:** 128–132. *pl. 12, 13 + f. 1, 2.* Je 1940.

Hoehne, F. C. O litoral do Brasil meridional. Excursão Bôtanica realizada de 16–9 a 26–10–1929, pela zona litorânea desde Santos até Laguna. Sec. Agr. Ind. e Com. Sao Paulo Dep. Bot. do Estado **4:** 1–111. *illust.* [1940].

Horovitz, S. Nuevo tipo de hibrido constante de trigo × centeno (*Triticum vulgare* × *Secale cereale*). Physis **18:** 285–290. *f. 1, 2.* 31 My 1939.

Hosseus, C. C. La Flora briológica argentina. Physis **15:** 1–12. 31 Mr 1939.

Howell, J. T. A new introduced grass in California. Leafl. West. Bot. **2:** 272. 18 Jl 1940.

Howell, J. T. Plants worthy of note—V. Leafl. West. Bot. **2:** 269–272. 18 Jl 1940.

Howell, J. T. Studies in *Ceanothus*—IV. Leafl. West. Bot. **2:** 259–262. 18 Jl 1940.

Hultén, E. The identity of the genus *Acroschizocarpus.* Bot. Notis. **1940:** 170–172. *f. 1.* 1940.

Hume, E. P. The response of plants to intermittent supplementary light. Proc. Am. Soc. Hort. Sci. **37:** 1059–1065. *f. 1.* My 1940.

Irénée-Marie, Frère. Contribution à la connaissance des Desmidiées du Québec. I–III. Nat. Canad. **67:** 98–112. *f. 1–8.* Ap–My 1940.

Jeffers, W. F. Studies on *Caryospora putaminum.* Mycologia **32:** 550–566. *f. 1, 2.* Jl–Au 1940.

Jenkins, A. E. & Bitancourt, A. A. An *Elsinoë* on *Toddalia.* Philip. Agr. **29:** 55, 56. Je 1940.

Joan, T. La *Tenia festoneada.* Physis **18:** 457–460. *pl. 11* + *f. 1, 2.* 31 My 1939.

Johnson, H. W., Lefebvre, C. L. & Ayres, T. T. Powdery mildew of *Lespedeza.* Phytopathology **30:** 620–621. *f. 1.* Jl 1940.

Johnston, I. M. The floristic significance of shrubs common to North and South American deserts. Journ. Arn. Arb. **21:** 356–363. Jl 1940.

Johnston, I. M. Studies in the Boraginaceae, XV. Notes on some Mexican and Central American species of *Cordia.* Jour. Arnold Arb. **21:** 336–355. Jl 1940.

Jones, C. H. Additions to the revised catalogue of Ohio vascular plants. VIII. Ohio Jour. Sci. **40:** 200–216. Jl 1940.

Jones, V. H. An ancient food plant of the southwest and plateau regions. El Palacio **44:** 41–53. 2–9 F 1938.

Jones, V. H. Some Chippewa and Ottawa uses of sweet grass. Papers Michigan Acad. Sci. **21:** 21–31. *pl. 3, 4.* 1936.

Kirk, E. Seven new genera of Carboniferous *Crinoidea inadunata.* Jour. Wash. Acad. Sci. **30:** 321–334. 15 Au 1940.

Kobuski, C. E. The Oleaceae of New Guinea collected by L. J. Brass. Jour. Arnold Arb. **21:** 328–335. Jl 1940.

Lackey, J. B. The microscopic flora and fauna of tree holes. Ohio Jour. Sci. **40:** 186–192. Jl 1940.

Lahitte, R. Gramineas nuevas o interesantes de la Flora argentina. Physis **15:** 305–310. *f. 1, 2.* 31 Mr 1939.

Laing, H. E. Respiration of the rhizomes of *Nuphar advenum* and other water plants. Am. Jour. Bot. **27:** 574–581. *f. 1–9.* Au 1940.

Lebedoff, G. A. Failure of cytokinesis during microsporogenesis in *Zea mays* following heat treatments. Cytologia **10:** 434–442. *f. 1–26.* Je 1940.

Legrand, D. Sistemática y Observaciones sobre las dos especies de *Portulaca* más comunes en el Uruguay. Physis **15:** 397–407. *f. 1–4.* 31 Mr 1939.

León, H. Contribución al estudio de las Cactaceas de Cuba. II. El *Leptocereus* de Cojimar. Mem. Soc. Cubana Hist. Nat. **14:** 133–137. *pl. 19, 20.* Je 1940.

Linder, D. H. Evolution of the Basidiomycetes and its relation to the terminology of the basidium. Mycologia **32:** 419–447. *f. 1–6.* Jl-Au 1940.

Lindquist, J. C. Dos Uredineas argentinas poco conocidas: *Bitzea Ingae* y *Uredo Ingae*. Darwiniana **4**: 129–132. *f. 1*. 25 Je 1940

Lindquist, J. C. Especies argentinas del género *Peronospora*. Physis **15**: 13–20. 31 Mr 1939

Ling, L. Factors affecting spore germination and growth of *Urocystis occulta* in culture. Phytopathology **30**: 579–591. *f. 1, 2*. Jl. 1940.

Livingston, R. & Franck, J. Assimilation and respiration of excised leaves at high concentrations of carbon dioxide. Am. Jour. Bot. **27**: 449–458. *f. 1–15*. Au 1940.

Long, W. H. & Goodding, L. N. Notes on *Gymnosporangium Cupressi*. Mycologia **32**: 489–492. *f. 1* Jl-Au 1940.

Looser, G. Las Pteridófitas del Parque Nacional de Nahuel Huapi. Physis **15**: 213–246. *f. 1–4*. 31 Mr 1939.

Louis-Marie, P. Le problème de nos dentaires hybrides. Ann. Acfas **6**: 101. 1940.

Luttrell, E. S. An undescribed fungus on Japanese cherry. Mycologia **32**: 530–536. *f. 1–10*. Jl-Au 1940.

McAlister, E. D. & Myers, J. The time course of photosynthesis and fluorescence observed simultaneously. Smithsonian Misc. Coll. **99**[3]: 1–37. *f. 1–16*. 28 Au 1940.

Mackenzie, K. K. North American Cariceae. Illustrated by Harry Charles Creutzburg. **1**: i–v, *pl. 1–269*. **2**: *pl. 270–539*, p. 540–547. The New York Botanical Garden, New York, 1940.

Madinaveitia, A. & Orozco D., F. Contribucion a la bioquímica del *Agave*. An. Inst. Biol. Mexcio **11**: 373–383. 1940.

Marchionatto, J. B. Las especies de *Cyttaria* y *Cyttariella* en la Argentina. Darwiniana **4**: 9–32. *pl. 1–7* + *f. 1–3*. 25 Je 1940.

Marchionatto, J. B. Notas micologicas. Physis **15**: 133–144. *pl. 1–9*. 31 Mr 1939.

Martin, G. W. The Myxomycetes. Bot. Rev. **6**: 356–388. Jl 1940.

Martinez, M. Pináceas Mexicanas. Descripción de algunas especies y variedades nuevas. An. Inst. Biol. Mexico **11**: 57–84. *f. 1–23*. 1940.

Martinez, M. Una nueva especie forestal (*Fagus mexicana* Sp. nova). An. Inst. Biol. Mexico **11**: 85–89. *f. 1–3*. 1940.

Mason, H. L. A pleistocene record of *Pseudotsuga macrocarpa*. Madroño **5**: 233–235. *f. 1*. 10 Jl 1940.

Merrill, E. D. Records of Indo-Chinese plants, II. Jour. Arnold Arb. **21**: 364–391. Jl 1940.

Meyer, S. L. M. Members of the genus *Phacus* Dujardin at Mountain Lake. Virginia Jour. Sci. **1**: 117, 118. My 1940.

Meyer, T. Nota preliminar sobre Gramineas forrajeras del Chaco. Rev. Argentina Agron. **7**: 95–104. Je 1940.

Milanez, F. R. Estudio anatomico do Lenho de trinta especies do genero *Aspidosperma*. Physis **15**: 429–490. *f. 1–39*. 31 Mr 1939.

Milbrath, J. A. *Coryneum* blight of oriental arborvitae caused by *Coryneum Berckmanii*, n. sp. Phytopathology **30**: 593–602. *f. 1–3*. Jl 1940.

Moldenke, H. N. The Eriocaulaceae, Verbenaceae, and Avicenniaceae of the Yucatan Peninsula. Carnegie Inst. Wash. Publ. **522**: 137–223. Je 1940.

Molle, C. C. Estructura anatómica del leno de las Ramnáceas argentinas del género *Condalia* (*piquillines*). Physis **15**: 409–420. *f. 1–8*. 31 Mr 1939.

Molle, C. C. Estudio histologico del tallo de la cascarilla (*Pogonopus tubulosus*). Physis **15:** 421–428. *f. 1–5.* 31 Mr 1939.

Monticelli, J. V. El genero *Larrea* Cavinelles. Su historia y revision. Physis **15:** 331–356. *f. 1–5.* 31 Mr 1939.

Moses, C. S. & Hoffmann, C. H. Isolation of *Ceratostomella ulmi* from *Scolytus multistriatus* adults stored at different temperatures. Phytopathology **30:** 701, 702. Au 1940.

Muenscher, W. C. Additions to our knowledge of the flora of Mount Baker, Washington. II. Madroño **5:** 236, 237. 10 Jl 1940.

Muller, C. H. A revision of the genus *Lycopersicon*. U. S. Dept. Agr. Misc. Publ. no. **382:** 1–29. *pl. 1–10.* Jl 1940.

Muller, C. H. The taxonomy and distribution of the genus *Lycopersicon*. Nat. Hort. Mag. **19:** 157–160. Jl. 1940.

Murneek, A. E. Some physiological factors in growth and reproduction of trees. Proc. Am. Soc. Hort. Sci. **37:** 666–671. My 1940.

Murray, M. J. The genetics of sex determination in the family Amaranthaceae. Genetics **25:** 409–431. *f. 1–3.* Jl 1940.

Mutinelli, A. Una interesante forrajera leguminosa indigena para Misiones (*Desmodium cuneatum* Hooker et Arnott). Physis **18:** 349–364. *f. 1–3.* 31 My 1939.

Nagel, C. M. & Leonard, O. A. The effect of *Cercospora beticola* on the chemical composition and carbon assimilation of *Beta vulgaris*. Phytopathology **30:** 659–666. Au 1940.

Neal, O. M. An extension of range of *Rynchospora macrostachya*. Rhodora **42:** 276. 15 Jl 1940.

Negroni, P. *Actinomyces discofoliatus* Grüter senelado por primera vez en la Argentina. Estudio micológico. Physis **15:** 146–157. *f. 1–11.* 31 Mr 1939.

Negroni, P. & Bonfiglioli, H. *Actinomyces israeli* (Kruse). Estudio micologico. Physis **15:** 159–192. *f. 1–19.* 31 Mr 1939.

Negroni, P. & Negri, T. Flora de *Aspergillus* del suelo de Buenos Aires. Physis **15:** 193–212. *f. 1–11.* 31 Mr 1939.

Niederhauser, J. S. & Whetzel, H. H. Observations on the varietal susceptibility of apples to *Gymnosporangium juniperi-virginianae*. Phytopathology **30:** 691–693. Au 1940.

O'Donell, C. A. La anatomia de una interesante planta áfila cuyana *Ramorinoa Girolae* Spegazzini. Physis **15:** 311–321. *f. 1–9.* 31 Mr 1939.

Ogloblin, A. A. Los pelos epidérmicos de la "yerba mate" (*Ilex paraguariensis* St. Hil.) Physis **18:** 459–466. *f. 1–4.* 31 My 1939.

Olson, R. A. & Buy, H. G. du. Factors influencing the protoplasmic streaming in the oat coleoptile. Am. Jour. Bot. **27:** 392–401. *f. 1–7.* 6 Jl 1940.

Overbeek, J. van. Traumatic acid and thiamin as growth factors for algae. Proc. Nat. Acad. Sci. **26:** 441–443. 15 Jl 1940.

Parodi, L. R. Estudio critico de las Gramineas austral-Americanas del género Agropyron. Rev. Mus. La Plata **3:** (bot.) 1–63. *pl. 1–12 + f. 1–21.* 27 My 1940.

Parodi, L. R. La distribución geográfica de los talares en la Provincia de Buenos Aires. Darwiniana **4:** 33–56. *pl. 1–13 + f. 1–4.* 25 Je 1940.

Parodi, L. R. & Pastore, A. I. Géneros de plantas cultivadas representados en la flora indigena de la Republica Argentina. Physis **18:** 255–268. 31 My 1939.

Pastore, A. I. Nota preliminar sobre las Equisetáceas argentinas. Physis **15:** 247–249. 31 Mr 1939.

Penfound, W. T. The biology of *Achyranthes philoxeroides* (Mart.) Standley. Am. Midl. Nat. **24:** 249–252. *f. 1, 2.* Jl 1940.

Penfound, W. T. The biology of *Dianthera americana* L. Am. Midl. Nat. **24:** 242–247. *f. 1–3.* Jl 1940.

Peto, F. H. & Boyes, J. W. Comparison of diploid and triploid sugar beets. Canad. Jour. Res. **18:** 273–282. *f. 1, 2.* Jl 1940.

Petrak, F. & Sydow, H. Ueber die Gattung *Amerosporium* Spegazzini und ihre nächsten Verwandten. Physis **15:** 79–86. 31 Mr 1939.

Pittier, H. Notas dendrologicas de Venezuela. IV. Bol. Soc. Venezolana Ciene. Nat. **39:** 1–17. 1939.

Plymale, E. L. Plants of Wayne County, West Virginia. Castanea **5:** 75–87. My 1940.

Pontis, R. E. El "Marchitamento" del pimiento (*Capsicum annuum*) en la Provincia de Mendoza. Rev. Argentina Agron. **7:** 113–127. *pl. 10, 11 + f. 1.* Je 1940.

Powell, G. M. Additions to the annotated catalogue of the vascular plants of Benton County, Washington. Northwest Sci. **13:** 72. Au 1939.

Pratt, R. & Fong, J. Studies on *Chlorella vulgaris* II. Further evidence that *Chlorella* cells form a growth-inhibiting substance. Am. Jour. Bot. **27:** 431–436. *f. 1–6.* 6 Jl 1940.

Price, W. C. Comparative host ranges of six plant viruses. Am. Jour. Bot. **27:** 530–541. *f. 1, 2.* Au 1940.

Quincke, F. L. Interspecific and intergeneric crosses with *Hordeum.* Canad. Jour. Res. **18:** 372, 373. Au 1940.

Raper, K. D. The communal nature of the fruiting process in Acrasieae. Am. Jour. Bot. **27:** 436–448. *f. 1–10.* 6 Jl 1940.

Record, S. J. Some new names for tropical American trees of the family Leguminosae. Tropical Woods **63:** 1–6. 1 S 1940.

Record, S. J. & Hess, R. W. American timbers of the family Bignoniaceae. Tropical Woods **63:** 9–38. 1 S 1940.

Reynolds, P. K. & Fang, Mrs. C. Y. The banana in Chinese literature. Harvard Jour. Asiatic Studies **5:** 165–181. *pl. 1–3.* Je 1940.

Ringuelet, E. J. Asociaciones vegetales del Rincón de Viedma (Bahia de Samborombón). Physis **15:** 261–289. *f. 1–7.* 31 Mr 1939.

Ringuelet, E. J. Flora del Rincón de Viedma (Bahia de Samborombón). Physis **15:** 491–493. 31 Mr 1939.

Rivera M., I & Breton M., R. Estudio acerca del conocimiento de las plantas llamadas vulgarmente "maleza" o "hierbas malas". An. Inst. Biol. Mexico **11:** 103–127. *f.* 1–50. 1940.

Robbins, W. J. Effect of extracts of *Phycomyces* upon its development. Am. Jour. Bot. **27:** 559–564. *f. 1–6.* Au 1940.

Rollins, R. C. A new *Cardamine* from North Carolina. Castanea **5:** 87, 88. My 1940.

Rollins, R. C. Notes on some crucifers of Texas, Mexico and South America. Jour. Arnold Arb. **21:** 392–396. Jl 1940.

Rollins, R. C. On two weedy crucifers. Rhodora **42:** 302–306. 13 Au 1940.

Rollins, R. C. Studies in the genus *Hedysarum* in North America. Rhodora **42:** 217–239. *pl. 597.* 15 Jl 1940.

Rosa Mato, F. Agaricales del Uruguay. Physis **15:** 123–127. *pl. 1–3.* 31 Mr 1939.

Rosa Mato, F. Hongos comestibles y venenosos. Physis **18:** 395–414. *f. 1–4.* 31 My 1939.

Rosa Mato, F. Las especies del género *Lepiota* en el Herbario Spegazzini. Physis **15:** 113–122. 31 Mr 1939.

Rossbach, G. B. & Rossbach, R. P. Southern occurrences of *Allium crenulatum* and *Meconella oregana.* Madroño **5:** 240. Jl 1940.

Rousseau, J. Le type de l'*Acer saccharium* d'après Linné et Kalm. Ann. Acfas **6:** 102, 103. 1940.

Rousseau, J. L'identité de l'*Acer palmifolium* Borck. Ann. Acfas **6:** 103. 1940.

Sainsbury, G. O. K. *Barbula rostrata* R. Br. Ter., an invalid species. Bryologist **43:** 113, 114. Au 1940.

St. John, H. Hawaiian plants named by Endlicher in 1836. Hawaiian plant studies 8. Bishop Mus. Occ. Pap. **15:** 229–238. 20 My 1940.

St. John, H. *Ophioglossum, Rollandia,* and *Scaevola.* Hawaiian Plant studies 9. Bishop Mus. Occ. Pap. 15: 351–359. *f. 1.* 26 Je 1940.

Samano B., A. Algas del valle de Mexico. Parte II. An. Inst. Biol. Mexico **11:** 41–50. *f. 1, 2.* 1940.

Schaal, L. A. Variation in the tolerance of certain physiologic races of *Actinomyces scabis* to hydrogenion concentration. Phytopathology **30:** 699, 700, *f. 1.* Au 1940.

Schade, A. L. The nutrition of *Leptomitus.* Am. Jour. Bot. **27:** 376–384. *f. 1–5.* 6 Jl 1940.

Schlenker, F. S. Plant growth in culture solution and availability of ions absorbed on permutit and aniline black. Am. Jour. Bot. **27:** 525–529. *f. 1–5.* Au 1940.

Schweinfurth, C. A nomenclatorial transfer. Harvard Univ. Bot. Mus. Leafl. **8:** 188. 2 Au 1940.

Schweinfurth, C. A notable extension of range of *Epidendrum oncidioides* var. *gravidum.* Harvard Univ. Bot. Mus. Leafl. **8:** 187. 2 Au 1940.

Schweinfurth, C. Some nomenclatorial transfers among Peruvian orchids. Harvard Univ. Bot. Mus. Leafl. **8:** 181–186. 2 Au 1940.

Seaver, F. J. Photographs and descriptions of cup-fungi—XXXV. A new species of *Patella.* Mycologia **32:** 567–569. *f. 1.* Jl-Au 1940.

Seckt, H. Las algas aerófilas de la Argentina. Physis **15:** 129–131. 31 Mr 1939.

Seeler, E. V. Two diseases of *Gleditsia* caused by a species of *Thyronectria.* Jour. Arnold Arb. **21:** 405–427. *pl. 1–4.* Jl 1940.

Seibert, R. J. New names in *Cybistax* and *Tabebuia.* Tropical Woods **63:** 7, 8. 1 S 1940.

Setchell, W. A. Some trabeculate *Codiums* (including two new species). Proc. Nat. Acad. Sci. **26:** 443–448. *1 pl.* 15 Jl 1940.

Shantz, H. L. The relation of plant ecology to human welfare. (*In* Symposium on relation of ecology to human welfare—the human situation). Ecol. Monogr. **10:** 311–329. *f. 1–25.* Jl 1940.

Shear, C. L. Mycological notes. IV. Mycologia **32:** 541–549. Jl-Au 1940.

Simpson, G. G. Types in modern taxonomy. Am. Jour. Sci. **238:** 413–431. Je 1940.

Singleton, W. T. & Mangelsdorf, P. C. Gametic lethals on the fourth chromosome of maize. Genetics **25:** 366–390. *f. 1–5.* Jl 1940.

Skoog, F. & Thimann, K. V. Enzymatic liberation of auxin from plant tissues. Science **92**: 64. 19 Jl 1940.

Smith, A. C. Studies of South American plants. IX. New and noteworthy species from the northern Andes. Am. Jour. Bot. **27**: 541–547. Au 1940.

Smith, A. V. Some plants recently found in the coastal plain of Maryland. Rhodora **42**: 277–280. 13 Au 1940.

Smith, L. F. Factors controlling the early development and survival of eastern white pine (*Pinus strobus* L.) in central New England. Ecol. Monog. **10**: 373–420. *f. 1–8*. Jl 1940.

Smith, P. Correlations of pollen profiles from glaciated eastern North America. Am. Jour. Sci. **238**: 597–601. Au 1940.

Soriano, A. Un caso de viviparidad y hemidibiosis natural en la acelga salvaje. Rev. Argentina Agron. **7**: 133–139. *f. 1, 2*. Je 1940.

Steere, W. C. *Tortula* in North America north of Mexico. Bryologist **43**: 76–86. 7 Je 1940; 98–109. Au 1940.

Stevens, N. E. & Ayres, J. C. The history of tobacco downy mildew in the United States in relation to weather conditions. Phytopathology **30**: 684–688. *f. 1*. Au 1940.

Summerhayes, V. S. The genus *Ficus* (Moraceae) in southeastern Polynesia. Bishop Mus. Occ. Pap. **15**: 227, 228. 11 Mr 1940.

Svenson, H. K. Report of the local flora committee I. *Lespedeza*. Torreya **40**: 105–108. *pl. 1, 2*. Jl–Au 1940.

Swingle, C. F. Regeneration and vegetative propagation. Bot. Rev. **6**: 301–355. Jl 1940.

Tang, P. & Loo, S. Tests on after-effects of auxin seed treatment. Am. Jour. Bot. **27**: 385, 386. 6 Jl 1940.

Taylor, W. R. Marine algae of the Smithsonian-Hartford Expedition to the West Indies, 1937. Contrib. U. S. Nat. Herb. **28**[3]: 549–561. *pl. 20*. 1940.

Thomas, P. T. The origin of new forms in *Rubus*, III. The chromosome constitution of *R. loganobaccus* Bailey, its parents and derivatives. Jour. Genet. **40**: 141–155. *pl. 6 + f. 1–13*. My 1940.

Thomas, P. T. Reproductive versatility in *Rubus*. II. The chromosomes and development. Jour. Genet. **40**: 119–128. My 1940.

Thompson, H. C. Temperature in relation to vegetative and reproductive development in plants. Proc. Am. Soc. Hort. Sci. **37**: 672–679. My 1940.

Thompson, R. H. A second species of *Riella* in North America. Bryologist **43**: 110, 111. Au 1940.

Timonin, M. I. The interaction of higher plants and soil microorganisms. I. Microbial population of rhizophore of seedlings of certain cultivated plants. Canad. Jour. Res. **18**: 307–317. *f. 1–3*. Jl 1940.

Tortorelli, L. A. Estudio tecnologico de las maderas de las especies argentinas del genero *Jacaranda* (Bignoniaceas). Physis **15**: 387–396. *f. 1–5*. 31 Mr 1339.

Troncoso, N. S. Las especies de Verbenáceas cultivadas en Buenos Aires. Physis **18**: 367, 368. 31 My 1939.

Tyner, L. E. The effect of crop debris on the pathogenicity of cereal root-rotting fungi. Canad. Jour. Res. **18**: 289–306. *f. 1, 2*. Jl 1940.

Vestal, P. A. & Vestal, M. R. The formation of septa in the fiber-tracheids of *Hypericum Androsaemum* L. Harvard Univ. Bot. Mus. Leafl. **8**: 169–180. *f. 1–9*. 2 Au 1940.

Villagran P., F. Contribuciones al conocimiento de la histologia y citologia del Maguey (*Agave*). An. Inst. Biol. Mexico **11**: 91–102. *f. 1–5.* 1940.

Wahl, H. A. Chromosome numbers and meiosis in the genus *Carex*. Am. Jour. Bot. **27**: 458–470. *pl. 1, 2.+f. 1.* Au 1940.

Wallace, J. M. Evidence of passive immunization of tobacco, *Nicotiana tabacum*, from the virus of curly top. Phytopathology **30**: 673–679. *f. 1.* Au 1940.

Wellman, R. H. & Heald, F. D. The toxicity of certain chemicals in aqueous solutions to spores of *Pencillium expansum*. Phytopathology **30**: 638–648. *f. 1.* Au 1940.

Wiggins, I. L. New and poorly known species of plants from the Sonoran Desert. Contr. Dudley Herb. **3**: 65–88. *pl. 14–21.* 8 Au 1940.

Wiggins, I. L. A new annual species of *Sidalcea*. Contr. Dudley Herb **3**: 55, 56. *pl. 13.* 10 Jl 1940.

Wiggins, I. L. Taxonomic notes on the genus *Dalea* Juss. and related genera as represented in the Sonoran Desert. Contr. Dudley Herb. **3**: 41–55. *pl. 6–12.* 10 Jl 1940.